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ADRENALINE ESTER

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It has been previously reported that after the ingestion of adrenaline in man a substance showing the properties of an adrenaline ester is eliminated in the urine (Richter, 1940a, b). The formation of an adrenaline ester under these conditions has now been confirmed by pharmacological experiments and the properties of the ester have been further studied.

METHODS. *Purification of ester.* A dose of 10 mgm. adrenaline in 10 ml. 0.5 per cent acetic acid containing 50 mgm. glycine was taken by one of us (D. R., 76 kgm.) by mouth and urine was collected for 2 hours before and 4 hours after administration. In order to avoid interference from other pressor substances which might be present in the urine the specimens were subjected to the following process of purification:

The urine specimens were diluted to a concentration corresponding to 100 ml./hr. To 100 ml. diluted urine were added 35 ml. 25 per cent normal lead acetate. The precipitate was centrifuged off and the clear solution made alkaline (pH 9) with strong ammonia solution. The precipitate which formed on standing for 15 minutes was separated by centrifuging, suspended in 4 ml. water and decomposed by slowly adding 60 per cent sulphuric acid and shaking vigorously until acid to Congo Red. The lead sulphate was centrifuged off, leaving a clear solution containing the phenol esters. A portion of this solution (3 ml.) was then hydrolysed by heating for 30 minutes on a boiling water bath with 0.22 ml. concentrated sulphuric acid. After cooling, 0.1 ml. saturated sodium acetate solution was added and the solution was brought to pH 3 by adding 40 per cent sodium hydroxide. The solution was cooled by shaking in a bath of cold water during the addition of the sodium hydroxide and the pH was controlled by adding drops of the solution on a glass rod to drops of in-

dicator (Thymol Blue and Bromphenol Blue) on a glass plate. Care was taken to avoid making the solution alkaline. A dark coloured precipitate which formed in the solution was removed by centrifuging. The hydrolysed portion was diluted to 5 ml. and 3 ml. of the unhydrolysed portion were also diluted to 5 ml. In this way 4 solutions were prepared: solutions (A) hydrolysed and (C) unhydrolysed, from the urine after taking adrenaline, and solutions (B) hydrolysed and (D) unhydrolysed, from the normal urine before taking adrenaline.

Pharmacological tests. Spinal cats were prepared as described by Burn (1937). The movements of the nictitating membrane were recorded by an isotonic lever after removal of the eyeball in a cat anesthetised with chloralose.

The intestine was prepared by making two longitudinal slits at about 10 cm. apart, separating the muscle from the mucosa, inserting cannulae in

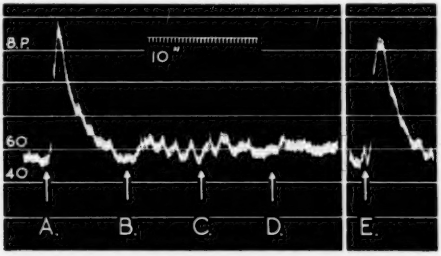

Fig. 1. Cat 3.8 kgm. spinal. Blood pressure response. A, 0.2 ml. solution from hydrolysed urine after adrenaline. B, 0.2 ml. solution from hydrolysed urine before adrenaline. C, 0.2 ml. solution from unhydrolysed urine after adrenaline. D, 0.2 ml. solution from unhydrolysed urine before adrenaline. E, Response to 2 μgm. adrenaline.

the free ends of the mucosal layer and recording the movements kymographically, as described by Babkin (1928). The adrenaline and other solutions were injected intravenously.

RESULTS. *Blood pressure of spinal cat.* The cat showed a clear blood pressure rise with 1 μgm. adrenaline. Neither of the solutions (B and D) obtained from normal urine, nor the solution (C) containing the adrenaline ester, had any pressor action; but the solution (A) containing the hydrolysed adrenaline ester gave a clear pressor response. The form of the pressor response to solution (A) was identical with that given by pure adrenaline (fig. 1).

The identity of the pressor substance in solution (A) with adrenaline was further confirmed by showing that it gave the characteristic reversal of the pressor action after administering ergotoxine.

Nictitating membrane and intestine. The nictitating membrane and

intestine showed a definite response to 1 μgm. adrenaline. The effects of the urine preparations on these structures are illustrated in figure 2.

With 1 ml. of the solutions prepared from urine before administering adrenaline (B and D) the response was negative. The response with unhydrolysed urine after taking adrenaline was only slight and corresponded to less than 1 μgm./ml. adrenaline, while solution (A) containing the hydrolysed ester gave responses corresponding to 25 mgm./ml. adrenaline. The slight response obtained with solution (C) containing unhydrolysed adrenaline ester corresponded to less than 5 per cent of that obtained after hydrolysis and may be attributed to slight hydrolysis at the ordinary temperature since it was not observed in every experiment.

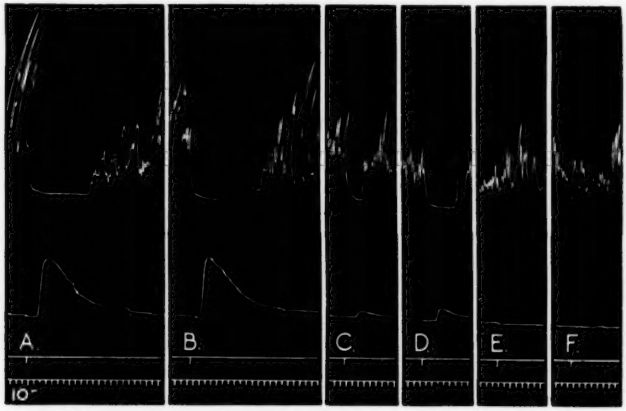

Fig. 2. Cat 2.5 kgm. female. Chloralose. Upper tracing movements of small intestine: lower tracing movements of nictitating membrane. A, 25 μgm. adrenaline. B, 1 ml. solution from hydrolysed urine after adrenaline. C, 1 ml. solution from unhydrolysed urine after adrenaline. D, 1 mgm. adrenaline. E, 1 ml. solution from hydrolysed urine before adrenaline. F, 1 ml. solution from unhydrolysed urine before adrenaline.

Rate of elimination of adrenaline ester. Quantitative estimations of the total amount of adrenaline ester eliminated could not be made on the purified solutions, since some of the ester was lost during the lead precipitations: an attempt was therefore made to estimate adrenaline directly in the urine after hydrolysis.

The urine specimens collected before and after taking adrenaline were diluted to 100 ml./hr. and hydrolysed by heating with sulphuric acid under the conditions described above. The adrenaline was then estimated quantitatively by the blood pressure in a spinal cat.

No appreciable amount of pressor substance was found in the hydrolysed urine collected before taking adrenaline: the amount found after taking

10 mgm. adrenaline corresponded to 6.1 μgm. adrenaline/ml. in the urine collected in the first 4 hours and 1.5 μgm./ml. during the subsequent 5 hours.

The total amount of adrenaline found in the urine was 2.4 mgm. in 4 hours and 3.2 mgm. in 9 hours or 32 per cent of the amount administered. The recovery of adrenaline in this experiment was lower than was found in previous experiments using the chemical methods of estimating adrenaline (Richter, 1940a); but the elimination was probably not complete in 9 hours and the rate of absorption of adrenaline may have been delayed in this case by the fact that the subject took a meal 55 minutes after taking the adrenaline.

DISCUSSION. The present experiments confirm the presence of an adrenaline-ester in the urine after the ingestion of adrenaline. The ester is pharmacologically inactive, or much less active than adrenaline, when tested on the blood pressure, nictitating membrane and intestine of the cat. This agrees with the conclusion that the adrenaline ester is an inactivation product.

Experiments *in vitro* have shown that adrenaline is readily oxidised by the enzymes catechol oxidase, amine oxidase and the cytochrome oxidase system (Green and Richter, 1937; Blaschko, Richter and Schlossmann, 1937; Richter, 1937) and it has been generally concluded that adrenaline is also inactivated *in vivo* by oxidation by one or other of these systems. It has been shown, however, that mammalian tissues do not contain an active catechol oxidase (Bhagvat and Richter, 1938), and further that the action of the amine oxidase is too slow to account for the observed rate of inactivation of adrenaline in the body unless it is assumed that the amine oxidase is much more active *in vivo* than *in vitro* (Richter and Tingey, 1939). Oxidation by the cytochrome system appears questionable when it is considered that apart from adrenaline many other substances such as hydroquinone, catechol, homogentisic acid and corbasil, which are readily oxidised by the cytochrome system *in vitro* are able to escape oxidation by this system *in vivo*, since they may be found to a considerable extent esterified or unchanged in the urine. The oxidation of adrenaline is certainly important in tissue preparations *in vitro* and is a factor that must be taken into account in biochemical and pharmacological experiments, but there is no evidence that adrenaline is oxidised *in vivo:* esterification is the only mechanism of inactivation for which there is, so far, any evidence, under normal physiological conditions.

Chemical evidence indicates that the adrenaline ester found in the urine is probably the sulphate ester, in which esterification has occurred at one of the phenolic hydroxyl groups. Such an inactivating mechanism would involve the "sulphosynthase" system, which is responsible for the synthesis of sulphate esters. This is an endothermic reaction which

requires that it should be coupled with an energy-producing reaction and that a source of energy such as a supply of oxidisable carbohydrate and oxygen should be present in addition to the sulphate. Inactivation by esterification will therefore depend on the general metabolic condition and the oxygen supply of the tissues as well as on the presence of the enzyme.

Analogy with other phenols suggests that the esterification of adrenaline occurs mainly in the liver, but it may occur also in other tissues since it has been shown that phenols are esterified after hepatectomy or even after evisceration (Marenzi, 1937; Barac, 1937). Further experimental work must be carried out before the significance of esterification in the inactivation of adrenaline liberated from the adrenals or from the sympathetic neurons under normal physiological conditions can be assessed.

SUMMARY

1. It has been confirmed that an adrenaline ester passes into the urine after adrenaline has been administered by mouth in man.

2. The adrenaline ester is pharmacologically inactive when tested on *a*, the blood pressure; *b*, the nictitating membrane, and *c*, the intestine of the cat.

3. Adrenaline liberated from the ester by hydrolysis shows the same properties as pure l-adrenaline when tested by these methods and shows the characteristic reversal in the pressor action by ergotoxine.

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STUDIES OF DIGESTION IN THE DOG

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The preparation of a re-entrant fistula. To combine the advantages of the various methods which have been employed for the purpose of collecting the contents of the gastro-intestinal tract for the study of digestion in dogs and at the same time to eliminate as many as possible of their disadvantages, a new type of preparation which has been called the "re-entrant fistula" has been developed. In general the method involves sectioning the intestine and inserting a silver cannula into the side of each of the blind stumps formed by closure of the cut ends. These cannulae are exteriorized and connected externally by a flexible rubber U-tube bearing a short side-arm. With this type of fistula the chyme simply flows from the upper to the lower segment of the intestine through the U-tube and no special care of the animal is required. Clamping the U-tube distal to the side-arm prevents passage of chyme beyond this point and permits recovery, from the side-arm, of the entire meal apart from what has been absorbed. Moreover, abnormal activity of the intestine caused by obstruction is obviated. This type of fistula is also useful in studies of absorption and secretion, and the visibility afforded by inserting a section of glass tubing into the rubber U-tube provides an excellent method for obtaining positive information concerning the mechanics of the digestive tract.

A general statement of the method has already been presented in abstract form (1) and the surgical procedure has been illustrated by Markowitz (2) but no description of technical details has yet been published. Since the appearance of these interim reports the authors have had extensive experience with the preparation and have worked out detailed surgical and post-operative procedures which have greatly improved the ease of preparing and maintaining the animals. These are reported in this paper. Some results of experiments are also given to indicate the normality of the preparation.

Nulliparous female dogs about one year old have been found to be the most satisfactory. It is advisable to clip the entire coat of hair and to withhold food for 18 hours before the operation, which must be performed under as rigidly aseptic conditions as obtain in human abdominal surgery. The procedure is as follows:

Stage 1. Laparotomy is carried out by the usual technique and a segment of the intestine is packed off.

Stage 2. After tying off the mesenteric vessels the gut is transsected between straight Carmalt forceps (i.e., clamps with longitudinal grooves). The cut ends, mobilized somewhat by dividing the mesentery for a short distance between them, are closed by the Parker-Kerr method.

Stage 3. An incision is made along the anti-mesenteric border of the upper stump and a Murphy purse-string laid down. When the cannula has been inserted, the purse-string is drawn tight and tied. It has been found advantageous to pass the tied ends of the purse-string back around the cannula to be tied again, thus further securing the cut edges of the incision to the cannula. A second purse-string, when drawn tight, invaginates the first line of suture effectively. The omentum is bisected between ligatures. The right half is folded on itself and the upper cannula passed through the center of this double layer. Four suspension sutures, superior, inferior, right and left, passing through the omentum and into the muscularis mucosa, are placed 1 cm. from the cannula. The lower stump is dealt with similarly.

Stage 4. A trocar is used to exteriorize the cannulae which are placed to the right of the mid-line about 7 cm. apart in the same sagittal plane, with the upper cannula clearing the ribs comfortably. A Reverdin needle is invaluable in exteriorizing the suspension sutures. It should be emphasized that these suspension sutures must be tied lightly. The abdomen is closed by the usual technique. The outer flanges of the cannulae are screwed on and the cannulae are connected by a flexible rubber U-tube. Gauze is packed about the tubes and a protective harness is put on the animal.

The purpose of the harness is to maintain in position a celluloid shield which protects the cannulae and rubber tube. The shield, 10 cm. long, 6 cm. wide and 6 cm. deep and perforated by numerous holes to allow free circulation of air, is sewn securely to the harness. The harness proper, of double thickness factory cotton, is provided with straps and buckles for ease in adjusting it to the animal firmly enough to eliminate movement of the shield.

Special requirements. Each cannula is of solid silver and weighs about 30 grams. The barrel is 4 cm. long with an internal diameter of 10 mm. and an external diameter of 12 mm. It is threaded right down to the internal flange. The latter is oval, 1.8 cm. in its narrowest and 2.7 cm. in its widest diameter. The outer flange is circular, 2.7 cm. in diameter and 5 mm. thick in the center, thinning to about 1 mm. at the edge. It is threaded to screw on to the cannula. The above measurements are those for cannulae to be used in the upper jejunum.

The trocar is a piece of tapered brass rod 6 cm. long. The cutting end,

1 cm. in diameter, has three edges coming to a point. The base, 1.5 cm. in diameter is drilled and tapped so that it can be screwed on to the cannula by a few turns after the former has been forced through the body wall. By pulling the trocar through, the cannula follows and is thus very easily exteriorized and a good snug fit ensured.

The flexible rubber tube connecting the external ends of the cannulae is constructed as follows. A glass form of the desired shape is dipped several times in liquid latex. When cured by gentle heating, a very strong rubber tube results, which is U-shaped, with a short side-arm. This latter is closed by a removable glass bung.

Post-operative care. The harness is removed twice a day. The incision is gently washed with warm soapy water, followed by 65 per cent alcohol and the gauze dressing is changed. If the outer flanges of the cannulae cut into the skin, a large flexible rubber washer is inserted between skin and flange. A carbolized glycerine solution is effective in clearing up minor skin infections. After 3 to 4 days the dog is allowed to lick the wound while under observation. An inclination to bite the tubes can sometimes be discouraged but the tendency, if present, is usually incorrigible. If the tubes are well tolerated the harness is left off during the daytime at first, and later discarded altogether. The median incision usually heals completely in 10 days, after which the animals look after themselves, keeping the abdomen clear of any exudate appearing around the cannulae.

Glucose (10 per cent) in normal saline (20 cc. per kgm. twice a day) is given intravenously for the first 4 to 5 days. The glucose and saline may then be given by mouth and milk substituted in gradually increasing proportions. About the tenth day, a little minced beef heart may be added and by the 14th day the animal should be on a full diet of minced beef heart (1 lb. per day) and milk (1 pt. to 1 qt. per day). This has been found to be an excellent maintenance ration, no vitamin or mineral supplements being needed.

The animals are kept in clean cages and reasonably warm (70°F.). Moderate exercise in the sun and open air aids in keeping them healthy. They are prevented from eating hair, bones, sawdust or pebbles and must not be fed raw vegetables, shredded wheat, dog biscuit or any similar granular material. Such substances tend to pack and plug the cannulae and may ruin the preparation.

The operation is not difficult and once the technique has been mastered operative mortality is negligible. Peritonitis has been encountered only once in the last twenty-eight consecutive preparations. Abdominal catastrophes, viz., intussusception, volvulus, portal thrombosis and acute enteritis are rare complications. When local infection, obstruction or excessive movement of the tubes leads to leakage of intestinal juice along

the cannulae, rapid digestion of the surrounding tissue takes place and such animals usually become useless. Some dogs will bite the U-tube at every opportunity, particularly those which are difficult to handle or those which have had litters. Others, e.g. Dalmatians, appear to be constitutionally unsuitable for this type of work. In a large series, the average duration of life post-operatively has been about two and one-half months. Several preparations have survived four months. This survival period, although not as long as might be desired, has made it possible to perform between twenty and twenty-five separate experiments per dog. Ultimately, in these preparations, one or both cannulae have come out of the intestine although still held in the abdominal wall and connected by a tunnel of fibrous tissue to the intestine. Subsequently the dense fibrous

TABLE 1

The time of disappearance of the x-ray shadow of a meat-barium sulphate meal in operated and unoperated dogs

DOG NUMBER	CONDITION	MEAL	TIME OF DISAPPEARANCE OF SHADOW FROM STOMACH AND SMALL INTESTINE
3	Operated	100 grams beef heart + 65 grams $BaSO_4$	3 hours 30 min.
3	Operated	100 grams beef heart + 65 grams $BaSO_4$	4 hours 0 min.
3	Operated	100 grams beef heart + 65 grams $BaSO_4$	4 hours 0 min.
18	Operated	100 grams beef heart + 65 grams $BaSO_4$	3 hours 10 min.
I	Unoperated	100 grams beef heart + $BaSO_4$	4 hours 0 min.
II	Unoperated	100 grams beef heart + $BaSO_4$	2 hours 30 min.
III	Unoperated	100 grams beef heart + $BaSO_4$	3 hours 45 min.
IV	Unoperated	100 grams beef heart + $BaSO_4$	3 hours 45 min.

tissue contracts and soon completely blocks the passage from gut to cannula.

Evidence has accumulated which indicates that animals thus prepared may be considered normal. Clinically they are perfectly well. Food is taken greedily, considerable weight is gained and a new coat of hair is quickly grown. The dogs are friendly and energetic. They exhibit marked alteration in behavior if they become ill. The time of disappearance of the x-ray shadow from the stomach, after feeding meat mixed with barium sulphate, does not differ significantly from that of unoperated dogs as shown by data in table 1.

There is, furthermore, no apparent evidence of disturbed function when freshly killed operated and unoperated dogs are compared as to the distribution and nature of the gastro-intestinal contents.

The animals can be used quite frequently for the collection of chyme. No depletion of blood electrolyte was observed in the prepared animals

even though chyme (100–300 cc.) was collected every other day, the CO_2-combining power of their blood being the same as that of the blood of unoperated dogs taken the same time after the feeding of identical meals.[1]

The gastric and duodenal digestion of protein. In order to study digestion *in situ* under as normal conditions as possible, dogs were prepared with the re-entrant fistula described above. The term digestion is defined in this study as the extent of hydrolysis of ingested foodstuffs, and is determined by analysis of the chyme collected. In the experiments recorded the fistulae were placed eight inches below the ligament of Treitz.

Preparation of material for feeding. In some preliminary experiments, meats as obtained from the butcher were first coarsely minced and then mixed with 300 cc. of water per pound, heated to 60°C. and allowed to cool. For fish meals, fresh unminced fillets were cooked for a short time in a double boiler to make them more palatable to the dogs. One hundred gram samples of each meat or fish so prepared constituted a meal. Although the weight and bulk of the meal were kept constant, the nitrogen and fat content varied considerably. Campbell (3) has shown that different kinds of meat and fish may contain varying proportions of nitrogenous extractives and therefore may stimulate the secretion of juices which differ in kind and amount, depending on the meat fed. Fat- and extractive-free proteins were therefore prepared from each type of meat and fish, and fed in quantities containing equal amounts of nitrogen. The effects on protein digestion of the separated fatty and nitrogenous extractive constituents were also investigated.

The proteins were separated as follows. The material was desiccated at 55°C. and at 2 to 3 cm. mercury pressure for a period of three days. The dried[2] meat or fish was then extracted with petroleum ether (B.P. 30–60°) in a large Soxhlet for 100 hours. The material removed by ether, when freed from solvent, constituted the "ether soluble fraction." The ether-extracted meat or fish was then extracted with 95 per cent ethyl alcohol for a further 65 hours. The material removed by alcohol, when freed from solvent, constituted the "alcohol soluble fraction." The ether and alcohol extracted meat or fish, after being freed from solvents by prolonged evacuation (17 mm. Hg) at 100°C., was ground in a mill and put through a 60-mesh sieve. The final product was designated "protein powder."

Collection of chyme. When chyme is to be collected the U-tube connect-

[1] The authors are very much indebted to Dr. Jacob Markowitz of the Department of Physiology, University of Toronto, for his unsparing effort and time in working out the surgical technique for this operation. They also gratefully acknowledge the invaluable suggestions of Prof. B. P. Babkin, and the assistance of Dr. Lionel Farber in making the determinations of CO_2-combining power of the blood.

[2] We are indebted to the Connaught Laboratories, University of Toronto, for the use of their large vacuum ovens.

ing the two fistulae is clamped distal to the side-arm (fig. 1) and the total output of chyme is collected in a flask kept at −20°C. by means of an ice-salt mixture. Freezing the chyme in this way has been found to arrest promptly further hydrolysis. When collected under these conditions it was observed that the consistency of the chyme, discharged directly from the U-tube, was at first quite fluid but it became progressively less fluid until finally it flowed with difficulty into the collection flask. When, however, a standpipe (fig. 1) was connected to the side-arm of the U-tube

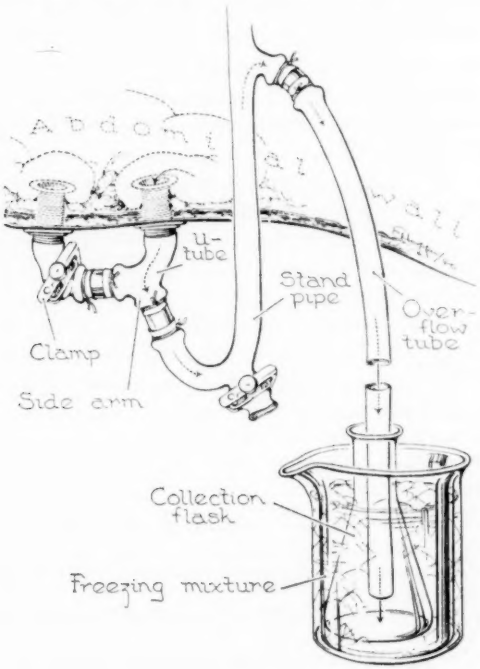

Fig. 1

to provide for the exit of chyme from the intestine under the hydrostatic pressure (about 5 in. of water) which exists in the intestine, it was found that the consistency of the chyme throughout an experiment appeared to be the same as from a meal not collected, but allowed to pass uninterruptedly through the U-tube. Furthermore, the time taken for the passage of the meal was the same. The "time for passage" of a meal is defined as the interval between the time the dog begins to eat the food (usually ingested in 1–3 min.) and the time when the last of the meal has passed the fistula. When the passage of the meal is nearly complete, the number

and size of the food particles gradually become less and peristaltic activity decreases. Then quite suddenly active peristalsis begins again and a few cubic centimeters of clear yellow juice and strings of mucus appear. Finally all activity suddenly ceases and is not resumed until more than an hour has elapsed. This phenomenon always occurs and is very easy to observe. The moment when activity ceases is taken to be the end of the passage of a meal.

In order to be sure that these dogs did not exhibit digestive idiosyncrasies, the extent of digestion and time of passage of a standard meal of haddock were determined in each dog before the animal was used in other

TABLE 2

Comparison of different fresh meats with respect to the extent of their digestion and to the "time taken for passage" in the gastro-intestinal tract

MEAL	NUMBER OF DOGS	NUMBER OF EXPERIMENTS	FAT IN MEAL	AVERAGE TIME FOR PASSAGE OF MEAL	AVERAGE EXTENT OF DIGESTION (TRICHLOROACETIC ACID NON-PRECIPITABLE NITROGEN IN CHYME)*
				Extreme values are given in parentheses	
			per cent	*hours*	*per cent*
Haddock, 100 grams (3300 mgm. N)	4	13	1	3 ($2\frac{1}{2}$–$3\frac{1}{3}$)	58 (56–58)
Beef heart, 100 grams (1510 mgm. N)	1	3	12	9† ($7\frac{1}{2}$–$9\frac{1}{2}$)	59 (56–62)
Salmon, 100 grams (3460 mgm. N)	1	1	15	8	63
Lamb, 100 grams (2575 mgm. N)	1	2	18	$7\frac{1}{2}$ ($7\frac{1}{2}$, $7\frac{1}{2}$)	62 (62, 63)
Pork, 100 grams (2250 mgm. N)	1	2	22	11 ($10\frac{3}{4}$–$11\frac{1}{2}$)	63 (63, 63)

* The values for average extent of digestion have not been corrected for the non-protein N of the fresh meats.

† This figure, 9 hours, is the average time for passage in 10 experiments on 4 dogs.

experiments. No significant variation was observed in any of these animals. The extent of digestion of protein was determined by the percentage of total nitrogen not precipitable by 2 per cent trichloracetic acid. Nitrogen was determined by the macro-Kjeldahl technique.

EXPERIMENTAL RESULTS. Preliminary experiments were carried out with meals of different fresh meats and the data in table 2 summarize the results.

The differences in nitrogen and fat content of the meal have, as will be shown later, such profound effects on the course and extent of digestion that it is impossible to draw any definite conclusions from these data as to the relative extent of digestion of the proteins unless the above factors are

controlled. Accordingly, fat- and extractive-free proteins were prepared, as described above, for further studies of protein digestion.

The "time for passage" of a meal. The time for passage of a meal (table 2) is much longer in most cases than that indicated by x-ray studies. The time of disappearance of the x-ray shadow from the stomach and small intestine, after feeding meat mixed with barium sulphate, is the same for both operated and unoperated dogs (table 1) and it was therefore concluded that these re-entrant fistula animals have normal gastro-intestinal function, but it was observed that the time for passage of a meal of 100 grams of minced beef heart, as determined by direct observation of the chyme in the U-tube, was approximately 9 hours, although the barium shadow, when barium had been mixed with the meal, disappeared in approximately 4 hours. This observation suggested the possibility that the emptying time, as determined by x-ray methods, is the emptying time with respect to barium sulphate and is not necessarily the emptying time for the material with which the barium was originally mixed. This possibility was tested quantitatively in the following way.

Haddock was fed to a dog and the chyme discharged during each hourly interval following the meal was collected in separate flasks. About 90 per cent (85–94) of the final total volume of chyme and amount of nitrogen was found to have been collected in four hours. When barium was fed with the haddock, and the dog examined under the fluoroscope, the shadow, as stated above, disappeared from the small intestine in four hours. This indicated that the time for passage of a haddock meal was fairly accurately indicated by the time for passage of the barium shadow. In the case of beef heart, however, only about 70 per cent (65–75) of the meal was collected in four hours. A further 4 to 5 hours were necessary before the remaining 30 per cent of the meal had been collected. In the case of a beef-heart meal, therefore, the time for passage was not accurately represented by the time of passage of the barium shadow which had been shown to be four hours. It seems evident that the movement of barium in the gastro-intestinal tract is not always an accurate indicator of the movement of food.

There are numerous references in the literature to the separation of different constituents of a mixed meal in the intestinal tract. Our results indicated that barium, at least in sufficient concentration to cast a shadow on fluoroscopic examination, had left the small intestine completely at a time when only 70 per cent of the nitrogen in the case of beef heart had passed through the stomach and duodenum. On the other hand, in experiments described later in which protein mixed with cellulose was fed, it was observed that the cellulose passed along more slowly than the protein. These observations suggest that different substances may indeed

be treated selectively with respect to the rate at which they are passed along the gastro-intestinal tract.

The digestion of protein in the presence of fats from different sources. In the experiments summarized in table 2 there were four variable factors: the amount and the type of protein and the amount and the type of fat. To determine whether protein digestion is affected by the *type* of fat present in the meal the first three of these variables were eliminated by feeding 100 grams of fresh haddock muscle (which contains only 1 per cent of fat), mixed with 22 grams of fat rendered from different types of meat.

These results (table 3) are in accordance with current opinion that the addition of fat retards the passage of a meal. They suggest, moreover, that the extent of retardation may vary with the nature of the fat. Furthermore, the figures given show that the digestion of protein was *increased* in the presence of fat. Although the retardation of passage of the meal in these experiments varied markedly from one fat to another, the extent of digestion of the protein varied only slightly. There is, therefore, no necessary parallelism between the extent of digestion and the "emptying time."

TABLE 3

The effect of fat on the digestion of 100 grams (3300 mgm. N) of protein (haddock muscle)

MEAL	NUMBER OF DOGS	NUMBER OF EXPERIMENTS	AVERAGE TIME FOR PASSAGE OF MEAL	AVERAGE EXTENT OF DIGESTION (TRICHLOROACETIC ACID NON-PRECIPITABLE NITROGEN OF CHYME)
			Extreme values are given in parentheses	
			hours	*per cent*
Haddock	4	13	3 (2½–3½)	58 (56–58)
Haddock + 22 grams beef heart fat	1	2	6⅔ (6½–7)	68 (67–69)
Haddock + 22 grams pork fat	2	4	9 (8½–9½)	66 (63–70)
Haddock + 22 grams lamb fat	2	3	10⅙ (8⅔–11¼)	64 (61–66)

Digestion of protein powders from different meats in the absence of fat. The digestion of proteins obtained from different sources at different levels of nitrogen intake was also studied. For these experiments protein powders, prepared as described above, were used. In calculating values for the extent of digestion allowance was made for the non-protein-nitrogen present in the powders. The results obtained are shown in table 5.

It appeared from these figures that fish proteins are digested to a somewhat greater extent than meat proteins under otherwise similar conditions.

Furthermore, this relationship was maintained when the amount of protein fed was increased over a wide range. Moreover, an increase in the amount of protein in the meal resulted in a decrease in the percentage of protein digested although the total amount digested was increased. It also appeared that although an increase in the amount of protein increased the time for the passage of a meal, this time showed surprisingly little variation from one protein to another if all were fed at the same level of protein intake.

Effects of the ether- and the alcohol-soluble fractions of mutton on the digestion of mutton protein. Experiments were performed to determine the

TABLE 4

Comparison of the extent of digestion and time for passage of protein powders from different meats at various levels of intake

MEAL (PROTEIN POWDER)	PROTEIN INTAKE	NUMBER OF DOGS	NUMBER OF EXPERIMENTS	AVERAGE TIME FOR PASSAGE OF MEAL	AVERAGE EXTENT OF DIGESTION (TRICHLOROACETIC ACID NON-PRECIPITABLE NITROGEN OF CHYME)
				Extreme values are given in parentheses	
	mgm. N			*hours*	*per cent of total N*
Beef muscle	1600	1	3	$2\frac{1}{2}$ ($1\frac{5}{6}$–$2\frac{3}{4}$)	36 (32–41)
	4800	1	3	$3\frac{3}{4}$ ($2\frac{3}{4}$–$5\frac{1}{6}$)	28 (21–34)
	9600	1	2	$4\frac{1}{6}$ ($3\frac{5}{6}$–$4\frac{1}{2}$)	22 (21–23)
Mutton	1600	4	7	$2\frac{1}{6}$ ($1\frac{1}{2}$–3)	26 (22–33)
	4950	1	2	3 ($2\frac{1}{3}$–$3\frac{5}{6}$)	20 (18–21)
Beef heart	1600	1	2	$3\frac{1}{4}$ ($3\frac{1}{4}$, $3\frac{1}{4}$)	25 (20–30)
Haddock	1600	2	5	$2\frac{5}{6}$ ($2\frac{1}{2}$–$3\frac{3}{4}$)	47 (42–57)
	4830	1	2	$3\frac{1}{2}$ ($3\frac{1}{2}$, $3\frac{1}{2}$)	38 (35–40)
	9660	1	2	4 ($3\frac{3}{4}$–$4\frac{1}{3}$)	24 (24–25)
Salmon	1675	3	6	3 ($1\frac{5}{6}$–$3\frac{1}{4}$)	44 (41–48)
	5130	1	2	$3\frac{3}{4}$ ($3\frac{1}{3}$–$4\frac{1}{6}$)	32 (31–34)
	10260	1	1	$5\frac{2}{3}$	24

effect, on the digestion of mutton protein, of adding the ether- and alcohol-soluble fractions of mutton to the meal. The results are given in table 5.

The most significant fact which emerged from these experiments was that the extent of digestion, the volume of chyme, and the time for passage of a meal were definitely modified by the addition of fat, extractives, or both. It is felt that more far-reaching conclusions should not be drawn until further work permits definite interpretation of these results. This degree of caution is necessary because the figures on per cent recovery prove that the chyme collected consists not only of fed protein but in addition an unknown and variable amount of nitrogenous material secreted into the intestine. This is discussed later.

Effect of increased bulk on digestion. It was found that the per cent digestion became less as the amount of protein nitrogen fed was increased. To determine whether the increased bulk of the meal was, in part at least, responsible for this, the effect of increasing the bulk of the meal without any increase in nitrogen was studied. This was accomplished by feeding protein powder intimately mixed with sufficient nitrogen-free cellulose to increase the bulk of powder fed six times.

It was evident that mere increase of bulk did not decrease but increased per cent digestion. Especially was this the case with the less digestible mutton. As might be expected, there was also increased absorption in seven out of ten experiments as indicated by the lower recovery of nitrogen. These results are in marked contrast with those of experiments (see table 4) in which the bulk of the meal was increased by feeding six times the amount

TABLE 5

The effects of ether- and alcohol-soluble fractions of mutton on the extent of digestion and time for passage of mutton protein (1600 mgm. N) in one dog (no. 16)

PROTEIN	NATURE OF MEAL		NUMBER OF EXPERIMENTS	TIME FOR PASSAGE OF MEAL	VOLUME OF CHYME COLLECTED	EXTENT OF DIGESTION	RECOVERY OF FED NITROGEN
	Ether soluble fraction added (fat*)	Alcohol soluble fraction added (extractives*)		Extreme values are given in parentheses			
	grams	*grams*		*hours*	*cc.*	*per cent*	*per cent*
Mutton powder			2	$1\frac{2}{3}$ ($1\frac{1}{2}$–$1\frac{5}{6}$)	135 (120–154)	24 (22–25)	96
Mutton powder	7*		3	$6\frac{2}{3}$ ($5\frac{1}{2}$–$8\frac{1}{6}$)	295 (280–310)	50 (48–52)	113
Mutton powder		0.79*	3	$1\frac{5}{6}$ ($1\frac{1}{2}$–2)	170 (158–190)	19 (18–20)	107
Mutton powder	7*	0.79*	2	$3\frac{1}{2}$ ($3\frac{1}{2}$, $3\frac{1}{2}$)	245 (222–275)	29 (27–31)	101

* These quantities are those which were obtained by extraction from the amount of fresh mutton containing 1600 mgm. of nitrogen.

of protein, where, as regards extent of digestion, the reverse result was obtained. Obviously the amount of proteolytic enzyme was the limiting factor for the extent of digestion in these circumstances.

pH of intestinal contents in the dog. Within the last few years it has been reported (4, 5, 6) that the pH of the chyme rarely rises above 7. The results of a few measurements on chyme collected just beyond the distal end of the duodenum are given below. All but one of these results fall within the range of pH 4.68 to 5.80.

The use of deuterium in following the course of protein digestion. An analysis of the chyme, for reasons already given, may not give a true picture of the course of digestion of an ingested protein. More nitrogen than has been fed is often recovered in the chyme (see table 5) and Babkin (7) and others have shown that the digestive secretions, in particular the pan-

creatic juice, may contain as much as 10 per cent protein. Accordingly, the total amount of nitrogen and the proportion of nitrogen in the protein

TABLE 6

The effect of increasing the bulk of a meal (by the addition of non-digestible nitrogen-free cellulose) on the extent of digestion and time for passage of protein powders

(One volume of protein powder (1600 mgm. N) was mixed with 5 volumes of cellulose.)

MEAL	DOG NUMBER	NUMBER OF EXPERIMENTS	AVERAGE TIME FOR PASSAGE OF MEAL	AVERAGE EXTENT OF DIGESTION (TRICHLOROACETIC ACID NON-PRECIPITABLE NITROGEN OF CHYME)	RECOVERY OF FED NITROGEN
			Extreme values are given in parentheses		
			hours	*per cent of total N*	*per cent*
Salmon	13	3	$2\frac{1}{2}$ ($1\frac{5}{6}$–3)	44 (42–45)	103
Salmon + cellulose	13	1	$3\frac{2}{3}$	56	97
Salmon	14	2	$3\frac{1}{4}$ ($3\frac{1}{6}$–$3\frac{1}{3}$)	43 (41–45)	73
Salmon + cellulose	14	3	$2\frac{3}{4}$ (2–$3\frac{1}{3}$)	63 (60–65)	25
Mutton	16	2	$1\frac{2}{3}$ ($1\frac{1}{2}$–$1\frac{5}{6}$)	24 (22–25)	9
Mutton + cellulose	16	4	4 ($3\frac{1}{2}$–$4\frac{2}{6}$)	66 (61–70)	49

TABLE 7

Electrometric measurements of pH of chyme collected from re-entrant fistula dogs

MEAL	DOG NUMBER	pH OF CHYME
100 grams fresh haddock	3	6.80
100 grams fresh haddock		5.65
100 grams fresh haddock		5.62
100 grams fresh haddock	5	4.68
100 grams fresh haddock		5.07
100 grams fresh haddock		4.90
100 grams fresh haddock	7	5.42
100 grams fresh haddock	8	5.70
100 grams fresh haddock		5.62
100 grams fresh haddock		5.15
100 grams fresh haddock + 22 grams lamb fat	7	5.80
100 grams fresh salmon	5	5.10
100 grams fresh lamb	7	4.70

and non-protein fractions of the chyme are dependent on three separate processes, digestion, absorption and secretion. Some idea of the amount of protein and non-protein nitrogenous substances present in the mixture

of gastric, pancreatic, biliary and duodenal juices secreted by the dog was obtained by collecting the secretions following a subcutaneous injection into a starved 20 kgm. dog of 0.75 mgm. of pilocarpine hydrochloride, or 0.5 mgm. of histamine hydrochloride. The nitrogen content of the digestive juices obtained amounted to from ten to thirty-five per cent of the nitrogen contained in protein which, if fed, would produce an equal volume of chyme. It is unnecessary to point out that the juice so obtained is probably not of the same composition as that secreted when food is fed.

The difficulty in interpreting results obtained by analysis of the chyme makes it necessary to seek a method of distinguishing between fed and secreted protein. In order to accomplish this it is necessary to "label" the protein fed. The possibility of using deuterium as a label in these experiments was investigated. Foster, Rittenberg and Schoenheimer (8), Krogh and Ussing (9), and Smith, Trace and Barbour (10) have shown that when mice are given heavy water to drink, deuterium is found in their tissue proteins. A proportion of this deuterium is incorporated in the protein molecule in such positions that its rate of exchange with ordinary hydrogen, on treatment with strong acid, is negligible; for Foster, Rittenberg and Schoenheimer (8) have obtained from the proteins of mice, hydrolysed by concentrated hydrochloric acid, ten amino acids containing stable deuterium.

It therefore seemed possible that if deutero-protein, so obtained, was fed to a fistulized animal, deuterium analyses of the protein and non-protein fractions of the chyme could be used to calculate deutero-protein nitrogen in these fractions. An accurate picture of the digestion of this protein would thus be obtained even though this digestion had taken place in the presence of unknown amounts of secreted nitrogen. It is necessary first to determine whether the rate at which deuterium appears in the non-protein fraction is a measure of the rate at which the protein molecule as a whole is being broken down. Accordingly, *in vitro* enzymic hydrolyses of deutero-protein were carried out to determine whether the appearance of deuterium in the non-protein fraction of the digest corresponds in a regular and reproducible manner with the appearance of nitrogen in the same fraction. An *in vitro* study of peptic hydrolysis of mouse-protein containing deuterium showed that deuterium analyses can indeed be used to follow the peptic digestion of deutero-protein. Further studies in which tryptic hydrolysis is carried out subsequent to peptic action, and of the general problem of protein digestion *in vivo*, have had to be postponed owing to preoccupation with war research.

SUMMARY

1. The operative technique of preparing a dog with the re-entrant fistula and the post-operative and subsequent care of the animals are described.

2. The prepared animals gave no evidence of disturbed function.

3. Some of the factors affecting the digestion of protein *in vivo* were investigated, using dogs with a re-entrant fistula situated just below the junction of the jejunum and duodenum. The effects of the presence or absence of fat and of nitrogenous extractives were studied, as well as the influence of "bulk."

4. Observations are reported which suggest that it is not justifiable to interpret "emptying time" (as obtained from x-ray studies) in terms of relative digestibility.

5. Errors in the determination of "per cent digestion" from an analysis of the chyme are discussed and a method which avoids these errors is proposed

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WARMTH SENSE IN RELATION TO THE AREA OF SKIN STIMULATED

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It has been shown (1, 2) that, within certain limits, the larger the area of skin exposed to a thermal stimulus, the lower is the intensity of stimulus necessary to evoke a sensation. This inverse relation between warmth threshold and area of skin exposed, which is a manifestation of spatial summation, has its counterpart in vision as established by Granit and Harper (3), and the recent experiments of Hartline (4). It must be pointed out that whereas the previous work on warmth sensation concerned itself with thresholds, the reports of Jenkins (5) indicate that the summation of super-liminal sensations does not follow the same summation law as does threshold sensation. This presents three possible relationships between sensation and size of the stimulated area: 1, a large sensation might be built up from a number of smaller ones by summation of the nerve impulses from several fibers; 2, the skin might become more sensitive as the result of addition of numbers of stimuli below the sensory threshold, that is, the threshold would be lowered; 3, both 1 and 2 might obtain. Possibilities 1 and 2 are not mutually inclusive, and although Hardy and Oppel have established the second, the recent work of Jenkins would deny the first. It is the object of this paper to present data which give a further analysis of the phenomenon of spatial summation of warmth establishing the summation of superliminal stimuli on the same basis as sub-threshold stimuli.

METHOD. The three methods used in a previous paper of this series (6) have been carried over in the present work and the reader is referred to that paper for a complete description and diagram of the apparatus. Briefly, the first method was as follows: the light from a 1,000 watt incandescent lamp was focussed upon an aperture behind which was the blackened forehead of the subject. In the light path was a manually operated shutter, and a half-sector disc which was rotated by a motor. Variable rheostats afforded control of the motor speed (and hence of the speed of rotation of the disc). The intensity of the radiation, which was measured with a radiometer, could be controlled by a variable rheostat.

Thus, the fusion frequency for warmth sense could be measured by observing the speed of the disc at which the sensory pulses fused into a continuous sensation. The area of skin exposed could be varied in discrete steps by placing shields, in which had been cut circular holes of the desired area, in front of the aperture through which the radiation flooded. For convenience, radiation intensities are reported in "units", one unit being 10^{-5} gram calories per second per square centimeter.

The fusion frequency for each area at constant intensity of illumination was determined for areas ranging from 1.0 to 27.3 sq.cm. of skin on the forehead. Typical curves are shown in figure 1, in which the fusion frequency has been plotted on a linear ordinate against the area on a logarithmic abscissa. The intensity for each test is given beside the curve. For a given subject, the greater the intensity of the radiation the higher

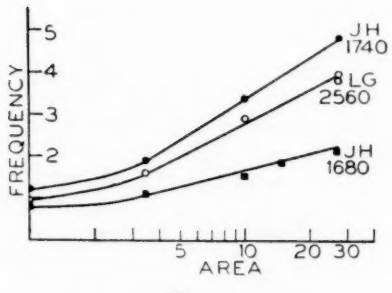

Fig. 1

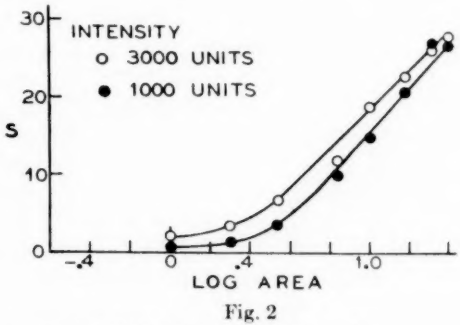

Fig. 2

Fig. 1. Fusion frequency (cycles/sec.) vs. area of skin stimulated (log scale).

Fig. 2. Reported sensation (S) relative to that from 27.3 sq.cm. taken as standard vs. log of area of skin stimulated. Intensity constant at 1,000 and 3,000 units.

was the level of fusion frequency as is demonstrated in figure 1 for J. H. L. G. differed from the other subjects in that he had generally lower levels of fusion frequency. The shape of all curves was essentially the same.

The second method of studying the dependence of sensation upon area was as follows: The sector was made to rotate at a constant frequency of 0.5 cycle per second, so that the heat pulse lasted for two seconds. The radiation intensity was set and kept constant at 3,060 units. The shutter was interposed between lens and aperture so that until it was raised, no radiation could reach the forehead. The subject then placed his forehead before the 27.3 sq.cm. area, and after a warning the shutter was raised and he was allowed to feel one full two seconds pulse of radiation. He was told to call the resulting sensation 10. The subject was then presented with radiation for two seconds through all the other areas (including the 27.3 $cm.^2$), selected at random, never knowing which area the operator had

chosen. A pause of about 45 seconds was allowed between each test. He evaluated the intensity of the sensation on the basis of that from the known 27.3 cm.^{2} Six subjects were thus three times tested in turn, being at all times unaware of their own score.

The three scores for each area were then totalled for each individual and the average total plotted against the logarithm of the area, as shown in figure 2. It is apparent that sensation depends upon area in the same way for large and small intensities of warmth.

The third method was that of determining the size of the area which gave a sensation just recognizably larger than that of a given area when the stimulus was held constant. This was done in the following manner: with the disc rotating so that the irradiation lasted two seconds, the subject was exposed through an aperture to a stimulus. He was asked to compare this sensation with another resulting from exposure to an area

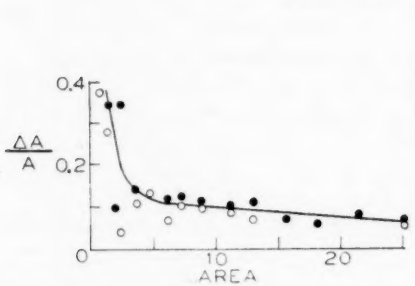

Fig. 3

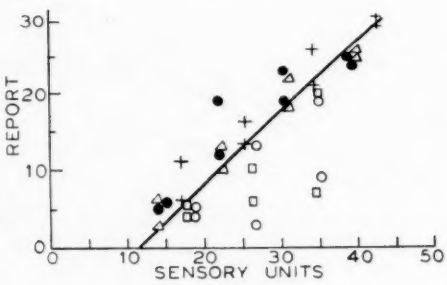

Fig. 4

Fig. 3. Plot of the ratio least distinguishable area difference/area vs. area.

Fig. 4. Ordinate gives report of relative sensation; abscissa is expected report calculated on basis of equation 5.

slightly larger (or smaller) than the first. The second stimulus was given about thirty seconds after the first. The area for the second stimulus was gradually changed by means of a calibrated iris diaphragm until the second sensation was just distinguishably larger (or smaller) than the first. The difference between the two areas was noted as Δ A. As might be expected, the same Δ A had to separate two areas, whether one increased from a smaller one or decreased from a larger one. The plot of Δ A/A as a function of the area is shown in figure 3. Δ A/A is a ratio comparable to the Weber ratio, Δ I/I, and is a measure of the sensory discriminatory ability. Of physiological significance is the change in Δ A/A as the intensity level of the sensation is changed by altering the size of the stimulated area. It is seen that the ratio Δ A/A is almost constant, when plotted against A, for areas larger than 3 to 5 sq.cm., but rises rapidly for the smaller areas. That is, for small areas the areal discrimination is relatively poor and

changes rapidly with change of area, whereas for the larger areas the percentage discrimination is better and is relatively constant.

COMMENT. It is apparent that the plot showing the reported increase in sensation as a function of area stimulated (fig. 2) is of the same form as that of the fusion frequency-area curve (fig. 1). The relationship in both cases is a logarithmic one for large areas with a definite break between 3 and 5 sq.cm. The similarity of the curves confirms the result of a previous paper (6) which stated that the fusion frequency can be used for a quantitative estimate of the intensity of the sensory experience of warmth.

The change in the direction of the curves of figures 1 and 2 occurring at about 4 sq.cm. is of interest because the spatial summation suddenly becomes greater for the smaller areas than would be expected from an extrapolation of the report for the larger areas. Figure 3, showing the plot of $\Delta A/A$, confirms the impression that something has occurred at about 4 sq.cm., for $\Delta A/A = 0.10 \mp 0.03$ from 5 cm.^{2} to 25 cm.^{2} and between 5 cm.^{2} and 1 cm.^{2} $\Delta A/A$ changes almost fourfold from 0.10 to 0.37, indicating a great proportionate decrease in the ability of the subject to distinguish changes in sensation caused by change in area. The observations all support the conclusion that spatial summation for the large areas is different from that for the small areas in that the degree of summation is greater in the small areas than in the large areas. This difference in summation is evident in the threshold values determined by Hardy and Oppel (5) who found 60 per cent summation for areas larger than 3.5 cm.^{2} and 75 per cent for the smaller areas. These two types of summation suggest two types of convergence of the sensory impulses from fibers subserving warmth. The smaller areas are probably served by endings whose branches converge near the periphery, whereas the larger areas contain summating fibers which do not converge until they have reached the central nervous system. It is known from Hartline's work that the first type of convergence is present in the eye, and Hardy and Oppel have shown that spatial summation occurs between the two hands, a convergence of the second type.

Hartline found that a stimulus either above or below threshold applied to one ending in the summative group caused an augmentation of activity in the ganglion cell when stimulated by other endings in its receptive field. Such a phenomenon does not occur with warmth sense in the second type of convergence, as is shown by the following experiment: The threshold for warmth was determined for an area of 13.5 sq.cm. on one side of the forehead by measuring the energy required to evoke a sensation at the end of a 3 second stimulus. The threshold was then redetermined for the same area while at the same time an equal area on the other side of the midline was constantly irradiated with a warmth stimulus. The results are shown in the table.

Normal threshold	Thresholds with accompanying irradiation	
55 ∓10 units	392 units	1200 units
	45 ∓10 units	90 ∓10 units

The thresholds in the first two columns are the same within the range of experimental error. The rise in threshold in the last case may be due to masking or to the distracting effect of the strong sense of warmth on the opposite side of the forehead. Thus, impulses arriving from one part of the summative field do not lower the threshold in another portion of the field if the convergence is of the second type. We have not investigated the first type of convergence for this effect. It appears, then, that spatial summation associated with the second type of convergence is not due to the lowering of synaptic resistances by other impulses which arrive at the point of convergence from other parts of the summative field. As this seems to be the case for the first type, it is apparent that the two types of summation are functionally different. The inference to be made from these results is that a single fiber together with its branches serves about 3 to 5 sq.cm. of skin on the forehead, and within this area one encounters the first type of summation. Convergence of fiber groups with larger areas is the second type of summation.

As has been noted before, the sensation vs. area curves for the larger areas are logarithmic. This fact can be expressed by the equation

$$S = k \log A \text{ (stimulus intensity constant)} \quad (1)$$

if S denote the intensity of the sensation, A the area, and k be a constant. It was previously shown that the dependence of sensory response on the stimulus intensity (I) follows a similar law, namely

$$S = k' \log I \text{ (area constant)} \quad (2)$$

The general equation must therefore be of the form

$$S = k' \log I + k \log A \quad (3)$$

This equation is exactly of the form as that obtained by Hardy and Oppel (2) in their work on warmth thresholds:

$$\log I_o + K \log A_o = \text{constant} \quad (4)$$

where I_o and A_o represent the particular conditions for threshold sensation. In fact, if one assumes that the right hand constant member of (4) contains the threshold sensation as a constant, then equations (3) and (4) become identical, and it is seen that the law for threshold sensation is obeyed by super-liminal sensations as well. It should, therefore, be possible to express the intensity of the sensory response evoked by any combination of area and intensity, within the range of validity of (3) and (4) in terms of the threshold sensation.

The formula which best fits the data of the sensory report vs. intensity curve is

$$S = S_0 (1 + b \log I/I_0)$$

and combining this with equation (1) gives (since the areal dependence of sensory response is of the same form as the intensity dependence):

$$S = S_0\left(1 + c \log \frac{IA^{0.78}}{I_0 A_0^{0.78}}\right)$$

In order to have this equation yield a curve parallel to the area-sensation report the constant c must be 29.2. Therefore, the equation expressing

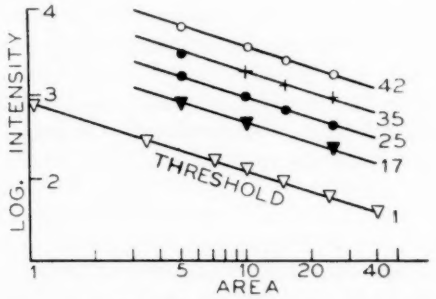

Fig. 5. Iso-sensory curves obtained by proper intensity-area combinations. Numbers beside curve give sensory units calculated from equation 5. Points on the curves were obtained from relative sensation tests.

the number of units of sensory response becomes, after substituting Hardy and Oppel's value of 778 for the $I_0 A_0^{0.78}$

$$S = S_0\left(1 + 29.2 \log \frac{IA^{0.78}}{778}\right) \quad \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

and this should be valid for the forehead for areas larger than 3 sq.cm. and intensities below 1,000 units. An equation of this type will apply to other parts of the body, but the constants will, of course, have different values.

A test of equation (5) was made. The method was analogous to that described above: a combination of I and A was chosen as standard and the sensory response from other combinations was evaluated in terms of this standard. The plot of the report of relative sensation against the sensory report calculated from equation (5) yields a straight line (fig. 4). Thus, the increase in sensation estimated from the subjects' judgments is in direct proportion to the increase in sensation calculated by the formula. This attests to the essential validity of the equation. If the log. of the area be plotted against the log. of the corresponding intensity, the combina-

tions being those for constant sensation, then one obtains a family of isosensory curves all of which are parallel to the curve obtained for thresholds by Hardy and Oppel. Such a plot is shown in figure 5 in which the number of units of sensation is the parameter.

SUMMARY

1. Further evidence of the reliability of the flicker method of studying temperature sensation has been outlined.

2. Two types of spatial summation for warmth have been found. One occurs peripherally, probably between branches of a single fiber; the other occurs centrally, between different fibers.

3. The second type of summation is not the result of a lowered synaptic resistance.

4. One fiber and its branches seems to serve about 3 to 5 sq.cm. of the forehead.

5. Areal discrimination of warmth is relatively poor at areas less than about 3 sq.cm. and is a rapidly changing function of area. For areas larger than 3 sq.cm., the relative discrimination is better and is practically constant.

6. A general expression for sensory response in terms of the threshold sensation has been obtained for the forehead for areas larger than 3 sq.cm. and intensities below 1,000 units. Super-liminal sensations are found to obey the same law as the threshold sensation.

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STUDIES IN EXERCISE PHYSIOLOGY

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Of the many types of bodily activity studied by physiological methods sustained heavy exercise is of particular importance because circulatory, respiratory and metabolic functions become the salient factors in individual performance. Provided the test exercise is simple and standard, skill and motivation are minimized and the true organic fitness of the individual is more surely assessed. Response to activity may, in the terminology of Briggs (5), be classed as: *a*, an overload in which the intensity of the activity precludes the attainment of a steady state; *b*, a crest-load in which the upper limits of the steady state are reached; *c*, a normal-load, any intensity of work within the range of capacity to maintain a steady state. The first two sections of this paper describe all three types of reaction but principally the last two. In the last section experiments are reported in which all three types of response were elicited.

I. *The effect of work-load and training on exercise heart-rate.* The behavior of heart rate during and after exercise and after a training period has been extensively studied. The level of heart rate during exercise has been shown by Bock and co-workers (3), among others, to be proportional to work-load. After a period of training, heart rate accelerates less for a given task. This has been noted by Steinhaus (17), Dill and Brouha (8) and Christensen (7). The present paper serves to give quantitative expression to these facts. Original and complete data may be found in the author's Doctoral Dissertation (18).

Two moderately trained subjects, R. M., age 23, and C. T., age 27, participated in 24 experiments each. The work, carried out on a bicycle ergometer equipped with an electric brake, was done at a pedalling rate of 65 r.p.m. but with resistances which gave work-loads varying from 636 to 1191 kilogram-meters per minute. Individual experiments were undertaken by the subject in the post-absorptive state, featured a constant work-load, and lasted from 45 to 50 minutes. Heart rates (HR) were counted approximately every five to six minutes with a stethoscope at the chest wall. Each subject was tested approximately three times a week over a period of two months. The work-loads were in the upper ranges of normal-loads and occasionally crest-loads, thus in a condition of relative steady state.

In general, the heart rate curve plotted against time shows an initial rise to about double the resting value in five minutes, a slower acceleration from 5 to 20 minutes, and a period of relative steady state from 20 to 45 minutes. Mean values for all experiments are as follows:

SUBJECT	WORK-LOAD	RESTING HEART RATE	HR AT 20 MIN.	HR AT 32.5 MIN.	HR AT 45 MIN.	SLOPE*
	Kgm. min.					
R. M.	941	63	147	152	157	0.40
C. T.	900	62	144.5	150.5	155.5	0.44

* Calculated by the method of least squares.

This shows that heart rate does not, in the typical case, reach an absolutely steady state but continues a slow upward drift throughout the 45 minute work period. This slope is largely independent of work-load and diminishes only slightly with training.

When heart rate (mean value from 20th to 45th min.) is plotted against work-load (WL) linear relationships are found for both subjects. The statistical values are:

SUBJECT	r (HR-WL)	S.E. (r)	REGRESSION EQUATIONS	S.E. (EST.)
R. M.	0.969	0.013	HR = 50.38 +0.106 WL	4.32
C. T.	0.955	0.020	HR = 79.22 +0.091 WL	4.12

Despite difference in the intercepts, substantial agreement is found between the two subjects in the other values.

Since these experiments were scheduled over a period of two months, a training effect was expected. Accordingly, partial regression equations were calculated including both work-load and days of training (D.T.). The values are:[1]

$$\text{For R. M.: } HR = 48.80 + 0.111\ WL - 0.222\ DT$$
$$\text{For C. T.: } HR = 58.68 + 0.115\ WL - 0.730\ DT$$

This disparity in regression coefficients for days of training is very likely due to the fact that R. M. was more physically fit than C. T. at the beginning of the series of experiments and hence would not be expected to show as great a training decrease.

[1] Partial regression formulae (Kendall and Yule, 11th ed., 1937, p. 285):

$$b_{yx \cdot z} = \frac{b_{yx} - b_{yz} \times b_{zx}}{1 - b_{xz} \times b_{zx}} \qquad b_{yx} = r_{yx} \times S_y/S_x$$
$$b_{yz \cdot x} = \frac{b_{yz} - b_{yx} \times b_{xz}}{1 - b_{zx} \times x_z} \qquad b_{yz} = r_{yz} \times S_y/S_z$$
$$b_{zx} = r_{zx} \times S_z/S_x$$
$$b_{xz} = r_{xz} \times S_x/S_z$$

II. *The relationship of circulatory and respiratory functions to work-load.* The central feature of physiologic response to exercise is an augmentation of vital functions to the point where adequate adjustment is made to the demands of the activity. If the intensity of the work undertaken is within the capacity of the subject to reach an approximately or absolutely steady state, circulation and respiration reach and maintain a slightly fluctuating

TABLE 1

Mean resting and work values

SUBJECT	WORK LOAD	HEART RATE	PULSE PRESS.	SYSTOLIC PRESS.	DIASTOLIC PRESS.	TOTAL VENT.	RESP. RATE
J. L.	Rest	53.8	35.4	105.6	70.2	5.8	10.1
	500	111.6	76.4	148.6	72.2	27.3	15.7
	600	132.2	80.3	158.5	78.2	30.4	15.8
	700	144.9	93.0	159.6	66.6	34.6	17.4
	800	163.3	128.3	193.7	65.4	39.3	18.6
V. B.	Rest	65.2	32.2	100.0	72.6	6.2	13.1
	500	124.5	32.9	117.1	84.2	27.5	18.2
	600	124.7	58.7	135.7	77.0	32.1	20.7
	700	144.0	48.8	125.5	76.7	36.0	20.7
	800	152.1	59.0	140.4	81.4	44.3	23.3
B. H.	Rest	52.6	42.8	116.2	73.6	10.3	11.9
	500	102.8	68.7	131.5	62.8	30.9	17.5
	600	108.6	70.8	133.9	63.1	34.6	20.3
	700	119.7	75.6	139.3	63.7	38.9	19.0
	800	130.9	90.3	158.0	67.7	45.2	21.7
M. S.	Rest	75.0	32.0	94.5	63.0	4.0	10.4
	273	133.2	38.0	104.0	66.0	15.9	19.0
	400	147.8	61.4	133.2	71.8	23.2	23.0
	500	155.7	63.3	134.3	69.0	28.7	25.0
	600	170.0	68.5	144.5	76.0	31.8	25.0
M. F.	Rest	65.5	28.3	91.0	62.8	5.1	13.0
	300	136.6	52.5	115.1	62.6	16.8	19.0
	400	138.2	48.4	112.1	63.7	14.4	22.1
	500	166.9	63.3	134.2	70.7	20.6	25.8
	600	189.0	63.2	136.2	73.0	17.3	35.3

plateau through the duration of the work. Within this range of work-loads, the steady state value of the various functions varies from individual to individual, but for a given subject is proportional to the intensity of the work. The general linear character of this relationship has been shown by the following workers: Heart rate, Tuttle and Wells (19), Christensen (6), and Bock et al. (3); systolic blood pressure, Gillespie,

Gibson and Murray (10); minute volume of blood flow, Bock et al. (3), and Christensen (6); oxygen consumption, Hill, Long and Lupton (11), and Christensen (6); total ventilation, Simonson (15), Bock et al. (3), and Hill, Long and Lupton (11). In addition to the results in section I of this paper the experiments reported here confirm these findings with quantitative data.

The subjects, ranging from 18 to 25 years of age, were moderately well trained students of physical education. One, B. H., was a college runner in active training at the time. Each subject undertook a series of work-loads, as indicated in table 1, experiments being spaced about a week apart. Resting determinations were made with the subject sitting upon the bicycle. All work readings were made during an uninterrupted experiment of 30 minutes' duration or until terminated by exhaustion. The male subjects found the work-loads well within their capacities, but both female subjects were exhausted and stopped about half-way through the last and most difficult experiments.

All experiments were carried out on a bicycle ergometer equipped with an electric brake. Work-load (WL) was measured in kilogram-meters per minute. Heart rate (HR) was counted with a stethoscope at the chest wall. Blood pressure was obtained by use of a special adaptation of the usual cuff and manometer system. A crystal microphone pickup and high-gain amplifier were used to detect the Korotkow sounds. The pressure at which sounds occurred was taken as the criterion of systolic pressure (SP) and the point of muffling of sound the diastolic (DP). Total ventilation (TV) was measured by collections of expired air in a 150 liter gasometer and respiratory rate (RR) by counting the falls per unit time of the bell of this instrument. The total number and time of readings were standard for all experiments.

A typical experiment is diagrammed in figure 1. After an adjustment period of variable duration the conditions of the steady state are met by most variables. All functions except heart rate tend to reach the plateau at not later than ten minutes and fluctuate somewhat about a horizontal curve. HR, however, as shown in the previous section, has a tendency toward a slow upward drift and does not typically reach an absolutely steady state.

In table 1 are given the mean values for the circulatory and respiratory functions at the corresponding work-loads. Data for the three male subjects were more numerous and the relationships more nearly rectilinear justifying the statistical computations shown in table 2. The correlations between work-load and heart rate, work-load and systolic pressure, work-load and total ventilation are significantly high since the probability of the correlations occurring by chance is in each case less than one in 10,000. This is also true for diastolic pressure and work-load in subject J. L. but

the same correlations in subjects B. H. and V. B. have P values of 0.0053 and 0.2077 respectively. The corresponding regression equations show high variability between subjects both in intercept and slope values. Diastolic pressure correlates positively in two cases with work-load, but negatively in the instance of subject J. L. This is in contrast to the tendency of all the other functions to increase with work intensity, although,

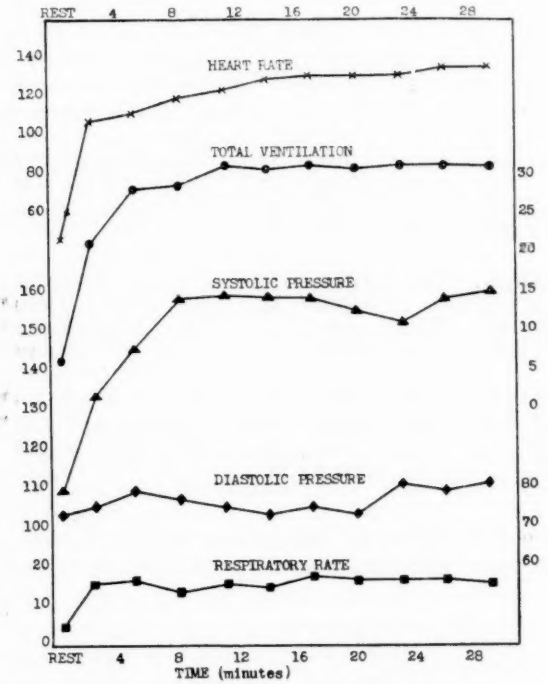

Fig. 1. The steady state. Subject J. L., pedalling rate 70 r.p.m., work-load 600 kilogram-meters per minute.

as noted in table 1, the departure of work values from the resting diastolic level seldom exceeds 10 mm. Hg.

The regression lines for pulse pressure (PP) were obtained by algebraic difference between the formulas for systolic and diastolic pressures. They are, for subject

J. L.: PP = 00.93 +0.1412 WL
V. B.: PP = 25.80 +0.0339 WL
B. H.: PP = 37.61 +0.0589 WL

Thus pulse pressure, following the trend of systolic pressure, increases linearly with work-load. The low intercept and high slope values for subject J. L. indicate a tendency for pulse pressure to rise with work-load more rapidly than usual. This may demonstrate in this subject comparatively low elasticity in the aortic artery and its branches. There was nothing inferior, however, in the general physical capacities of J. L. who has frequently been used as subject in experiments of this type.

TABLE 2

Statistical values: male subjects

SUBJECT	CORRELATION	r	S.E. (r)	N	REGRESSION EQUATION	S.E. (EST.)
B. H.	HR–WL	0.904	0.0323	32	HR = 67.66 +0.0776 WL	4.81
	SP–WL	0.859	0.0463	32	SP = 94.70 +0.0697 WL	5.44
	DP–WL	0.476	0.1366	32	DP = 57.09 +0.0108 WL	2.61
	TV–WL	0.907	0.0154	32	TV = 10.21 +0.0414 WL	2.52
V. B.	HR–WL	0.691	0.0313	36	HR = 97.44 +0.0585 WL	9.14
	SP–WL	0.611	0.0622	36	SP = 03.24 +0.0390 WL	7.50
	DP–WL	0.215	0.1590	36	DP = 77.44 +0 0051 WL	3.53
	TV–WL	0.915	0.0272	36	TV = 8.30 +0.0399 WL	2.60
J. L.	HR–WL	0.941	0.0184	39	HR = 45.47 +0.1386 WL	6.83
	SP–WL	0.904	0.0293	39	SP = 90.99 +0.1130 WL	7.79
	DP–WL	0.615	0.0997	39	DP = 90.06 −0.0293 WL	5.01
	TV–WL	0.843	0.0465	39	TV = 9.32 +0.0365 WL	3.40

These experiments were not designed specifically to study differences between the sexes but the following tabulation offers basis for such contrast:

SUBJECT	WORK-LOAD	WORK/ BODY WEIGHT	HR	SP	DP	TV	RR
		kgm./min./ kilo.					
V. B.....	600	9.72	144	135.7	77	32.1	20.7
M. F.....	500	9.26	167	134.3	69	28.7	25.8
M. S.....	500	9.26	155	134.2	71	20.6	25.5

The differences between male subject V. B. and the female subjects M. F. and M. S. are most marked in the cases of heart rate and respiratory rate although the female subjects performed a slightly lesser task. Since physically trained subjects generally display lower values in these functions the differences are probably only quantitative in nature.

The rapid and shallow breathing of subject M. F. at 600 kgm. per minute

was obviously inadequate to meet the demand for oxygen and the work, which terminated at eight minutes, was doubtless sustained by the accumulation of a large oxygen debt. M. F. was near collapse at the end.

III. *Circulatory, respiratory and metabolic responses to an increasing work-load.* In the foregoing experiments a constant work-load was maintained throughout and the response to a range of work levels inferred from a series of such experiments performed on different days. This section reports data from experiments in which a constant pedalling rate on a bicycle ergometer was maintained but with a periodically increasing resistance until exhaustion resulted. With this arrangement both the trend of adjustment to work of increasing intensity may be demonstrated and the physiologic events occurring on the approach to exhaustion may be elucidated.

The subjects participating in the experiments were all physical education students or men with athletic experience with the exception of A. L. who was completely untrained. All experiments were scheduled in the morning with subjects in the post-absorptive state. After resting determinations with subject seated on the bicycle, work was begun at a low level and periodically increased to the highest of which the subject was capable before succumbing to exhaustion. The pedalling rate was constant at 70 r.p.m. throughout. The time spent at each level was five minutes for series I, two minutes for series II and III, and from 1 to 5 minutes in the alveolar pCO_2 experiments. Total working time varied from 9 to 26 minutes, depending upon the capacity of the subject as well as length of step interval. Excellent coöperation was obtained from each subject, and while admittedly motivation may cause variation in the "exhaustion" endpoint, adequate evidence was found that in every case the stopping of work coincided with objective criteria of physiologic distress.

In addition to the methods used for the determination of circulation and respiration, explained in the second section of this paper, oxygen consumption, carbon dioxide production and the respiratory quotient were determined by collection and analysis of expired air. The original face mask was replaced by one patterned after the improved model of Enghof (9). Alveolar air samples were taken by the Haldane-Priestley technique according to the practice of Bock et al. (2). Gas samples were analyzed in a Haldane apparatus which routinely gave the following results with atmospheric air; oxygen, 20.93 vol. per cent ± 0.05 (S.D.), and carbon dioxide, 0.04 vol. per cent ± 0.01 (S.D.).

In series I the author endeavored to choose a work schedule such that each subject would have a low and an intermediate work-load for a "warm-up" and two or more steps at or near his capacity. The hoped for sampling of responses at the upper limit was not always adequate because of lack of a reliable method for predicting where that level would lie for a

given subject. This made for less uniformity in the data than is desirable. Accordingly, in the subsequent experiments, series II and III, a two-minute step was adopted, each subject working through an orderly progression of steps. While the ideal would require that work-load vary continuously with time, this was not feasible with the available apparatus. The conditions of the steady state were not met in this type of experiment but the orderliness of response in most cases, excepting at the exhaustion level, is evidence of a continuing adjustment to the demands of the work. Curves for subject A. L., who was a student of very sedentary habits, differ markedly from all other subjects. In the case of subject J. B. work was continued for four minutes at the uppermost level with two determinations being made during that interval. J. R., the subject of series III, was a very strong athlete, in a good state of training, and habitually rode a bicycle. The experiments diagrammed in figure 5 show the trend of alveolar pCO_2. Varying step durations were used, e.g., J. L. and C. T. five minutes, W. P. (A) one minute, and W. P. (B) two minutes. There are insufficient data here to assess the effect of rate of work increase (this is to be the subject of further study in this laboratory) but no very marked differences are apparent.

Heart rates in nearly all cases rise linearly with work-load throughout the low and intermediate range of work-loads. The position and degree of the slope is an individual variable which undoubtedly has much to do with the capacity of the subject. In general, although there are exceptions, the individuals of greatest capacity show lower heart rates per unit work. Steinhaus (17) has listed this as a characteristic of the trained state. For example, subject J. R. attained a maximal work output of 20 kgm-min./kilo of body weight with a heart rate of 197, subject A. L. only 8.6 with a rate of 250. Another factor, the "ceiling" of effective heart action, remains to be evaluated. Over a wide age range Dill and Brouha (8) have shown that this limit is by no means uniform. In our data nearly half of the cases demonstrate no change in the linear increase, forty per cent show an upward spurt and the remainder tend toward a plateau. In the absence of data on systolic output of the heart it is impossible to say at what heart rate cardiac output begins to suffer from inadequate filling time. Rates at or in excess of 190 probably are incompatible with effective heart output since in all cases where this level has been reached the subject was very near to exhaustion.

For series II, in which blood pressure determinations were made, the Liljestrand-Zander factor was computed to give an indication of the trend of circulation rate. This factor,[2] according to Christensen (7), is a useful index of the trend of cardiac output. Curves for this value (fig. 2) are

[2] L. Z. Factor $= \frac{\text{Pulse pressure} \times 100}{\text{Mean arterial pressure}} \times \text{HR}$

approximately linear with work-load in two cases while for the remaining two a slight upward concavity is apparent. On the approach to maximal levels, however, there is no manifest sign of circulatory failure accompanying exhaustion. Indeed, when the compilation of blood pressure and heart rate data in figure 6 is considered there is little evidence of gross circulatory failure as a cause of exhaustion of the subject, although it can not be excluded that failure of adequate blood supply to certain tissues may play an important part in the onset of exhaustion.

Total ventilation likewise displays a linear increase with work-load tending toward acceleration at maximal levels. The rate of increase varies

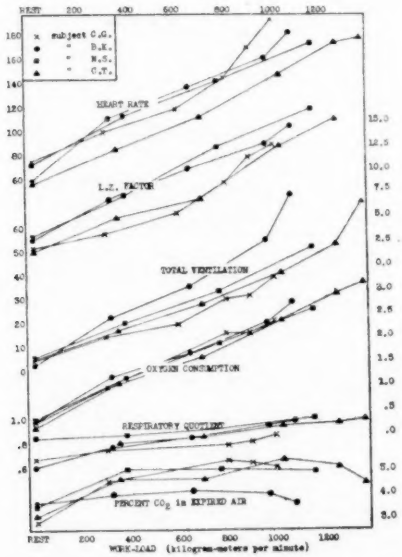

Fig. 2. Step-up experiments: Series I. Five subjects working to exhaustion on a bicycle ergometer.

considerably from subject to subject (figs. 2 and 3) but is fairly constant for the individual, as shown in figure 4. The ventilation per unit of work also varies with the subject. Extremes are again represented by subjects A. L. and L. S. (fig. 3). The lower ventilation of the latter subject is associated with the consistently high per cent of carbon dioxide and oxygen deficit in the expired air. On the approach to exhaustion, total ventilation (fig. 6) usually accelerates excessively. Final ventilations range from 40 liters per minute for subject L. S. to 115 for J. R.

The rate of oxygen consumption is a highly significant physiological variable not only because it represents the physiological cost of the work

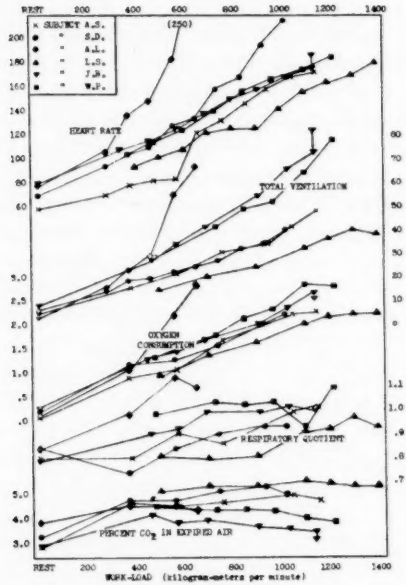

Fig. 3. Step-up experiments: Series II. Six subjects working to exhaustion on a bicycle ergometer.

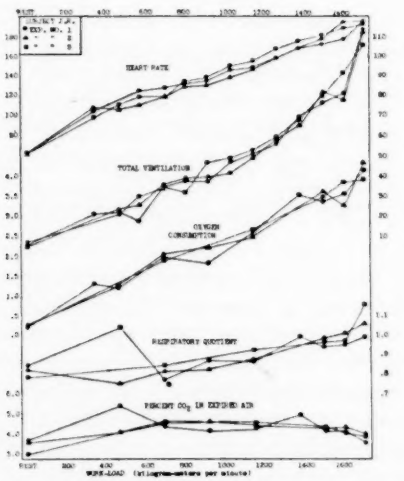

Fig. 4. Step-up experiments on subject J. R. Three identical experiments carried to exhaustion on one subject.

but because it gives evidence of the transport capacity of the circulatory and respiratory mechanisms. The position of the oxygen consumption curve relative to work-load is a measure of the efficiency of the subject. As will be noted later, the uncertainty of obtaining a true R.Q. under the conditions of these experiments does not justify the computation of Calories consumed and hence the precise calculation of net or gross me-

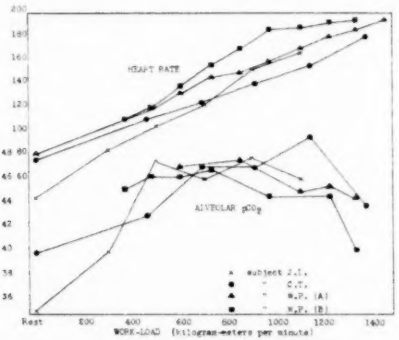

Fig. 5. Step-up experiments. Heart rate and alveolar carbon dioxide tension in four subjects in bicycle ergometer work increased to the point of inducing exhaustion.

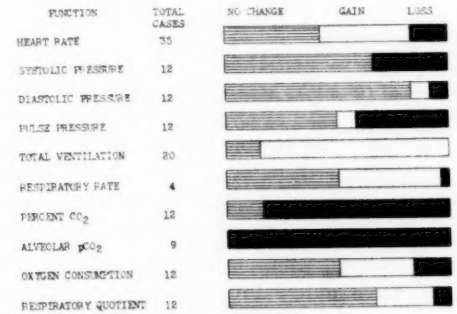

Fig. 6. Exhaustion trends. Trend of functions on approach to exhaustion levels of work. The graphs show per cents of all observations showing gain; no change, or loss in linear increase with work-load.

chanical efficiency. Subjects in series I have closely comparable efficiencies but more variability is found in series II where again subjects A. L. and L. S. represent the extremes of low and high efficiency. The trend of oxygen consumption at maximal levels is of great significance because of the prevailing view that the ability to absorb oxygen is a limiting factor in individual physical performance. As summarized in figure 6 oxygen consumption is by no means always deficient at exhaustion levels.

In fact, in 50 per cent of the cases no deviation in the linear increase of oxygen intake occurs at exhaustion and in the remaining cases this value may accelerate more often than fall off. In those cases where the curve turns upward at the approach to exhaustion the hypothesis may be advanced that the effectiveness with which the muscles are performing the work is lowered necessitating the mobilization of additional motor units in an effort to sustain the rate of work. This might readily be due to a "central" fatigue effect according to the theory of Simonson (16). That is, an alteration of the physico-chemical conditions of blood and tissue fluids acting upon central nervous centers might disturb the coördination of the muscular activity so that more muscle fibers might be required for the task.

The respiratory quotient obtained in step-up experiments is the resultant of two effects: *a*, the proportion of fat, protein and carbohydrate catabolized, and *b*, changes in the carbon dioxide content of the blood. A true R.Q., e.g., indicative of the concurrent metabolism, is obtained only when the body is in the basal state or the steady state of exercise. Several workers, notably Bock et al. (4), have shown that the true R.Q. rises with work-load, approaching unity at maximal work levels. This trend is evident in the present experiments but the erratic nature of some of the curves and quotients in excess of unity are ascribed to washing out of blood carbon dioxide reserves in the periodic transitions to higher work levels as well as the dyspnea occurring at exhaustion.

The per cent of carbon dioxide in expired air and the alveolar carbon dioxide tension both serve as indicators of blood pH fluctuations. In the transition from rest to work these values rise to a variable plateau which is maintained throughout most of the work range but on the approach to exhaustion show a fall. This latter fact is strikingly demonstrated in figure 6. The explanation for several features of exhaustion, namely, accelerated ventilation, dropping alveolar pCO_2 and per cent carbon dioxide in expired air, doubtless all have a common basis in a rapid accumulation of blood lactate. Determinations of alveolar tension, from rapidly obtained H-P samples, have more value in predicting the onset of exhaustion since the CO_2 in expired air is usually determined from collections of 2 to 5 minutes' duration and hence does not follow the change of state as closely.

Discussion. Schneider (13) carried out experiments on six subjects in which a series of five work-loads were attempted, each 6 to 8 minutes in duration separated by 20 minute rest periods. He found that the subjects maintained linear increases in heart rate, oxygen consumption, per cent of CO_2 in expired air, and total ventilation up to a certain load after which the first three functions tended to fail to maintain the increase while ventilation augmented excessively. The maximum work-loads at which these functions could maintain their linearity—the crest-load—varied both with the specific function and with the individual subject.

Schneider's subjects consumed oxygen at a rate proportional to work-load up to the crest-load beyond which one subject, R. J., showed an augmented consumption, another, W. C., maintained the linear increase, and the four remaining subjects failed to increase their oxygen consumption proportionally. The trend of heart rate above the crest-load was likewise variable. One subject, R. J., maintained the linear increase, but all others failed. The gain in acceleration found in about 40 per cent of our cases was not found in Schneider's experiments. The significance of the peak of per cent CO_2 in expired air as the criterion of crest-load, advocated by Briggs (5), may be questioned on the basis of Schneider's and our own data. For example, subject W. C. who was Schneider's fittest subject had a crest-load at 6000 ft-lb., judging by the per cent CO_2 in expired air, but was able to carry 8000 and 10,000 loads adequately. H. M. reached the same CO_2 percentage peak at 4000 and 6000 ft-lbs. In our own data the point at which the CO_2 percentage reached its peak is highly variable sometimes occurring at low work-loads and often at high levels. The general tendency of the curves (figs. 2, 3, 4) is toward a plateau higher than the resting level but maintained throughout the major range of work-loads. With the approach to exhaustion levels, however, the curves begin a decline, dropping in some instances below the resting level. It can be seen from figure 6 that this final drop in per cent CO_2 in expired air is second only to alveolar pCO_2 as a reliable index of the approach of exhaustion. If this function is followed closely and the subject pushed to his limit the same uniformity should be obtained as for pCO_2. It seems justifiable to conclude that this final dip which comes on rapidly with the approach of exhaustion is a better criterion of the subject's capacity for work short of exhaustion than the peak of the CO_2 percentage curve. Determinations of alveolar carbon dioxide tension (fig. 5) give an even better criterion of the breakdown in adaptation to work.

Crest-load oxygen consumption. The step-up experiment affords an opportunity to measure a type of individual capacity hitherto little explored. That oxygen consumption is an index of physiological cost of work has been stressed by Hill, Long and Lupton (11), and by Steinhaus (17). Furthermore, the ability to consume oxygen at a high rate has been shown by Christensen (6) and by Robinson et al. (12) to be an important feature of the response of the top-ranking athlete. Such a capacity to maintain a high oxygen consumption over a period of time demonstrates a large degree of cardio-vascular and respiratory fitness. Indeed, the rate of oxygen consumption in sustained work may be taken as a measure of these capacities, assuming that at least a relative approach has been made to the conditions of the steady state and that therefore the circulo-respiratory functions are in an adequate state of adaptation to the demands of the activity. These requirements are met in the step-up experiment if the

step preceding the exhaustion level is taken as the crest-load—as the highest level of activity which the subject can maintain without any of the extreme divergencies in function which occur at the exhaustion level.

In figure 7 eleven subjects are ranked according to the work output achieved before becoming exhausted. The two principal factors determining the capacity are graphed in such a way as to show the relative importance of each, and the relationship of the two combined (visualized from entire length of each bar) to the work output. The figures for efficiency were obtained from oxygen consumption and kilogram-meters of work, since the calculation of metabolism in calories was deemed inadvisable in the absence of a true steady state. It should be noted that with the ratio employed high values represent low efficiency and vice versa.

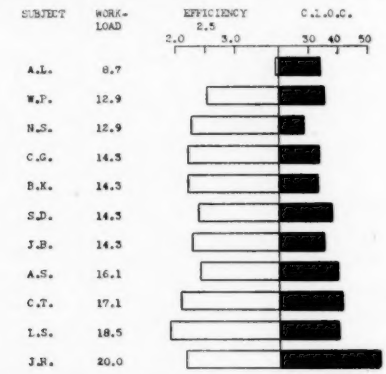

Fig. 7. Ranking of subjects on step-up test. C. L. O. C., oxygen consumption at the crest-load in cubic centimeters per kilogram; efficiency, oxygen consumption at the crest-load per kilogram-meter of work; work-load, rate of work at the crest-load in kilogram-meters per kilogram of body weight.

There is a linear increase in the sum of these two values with work-load and in general with each measure separately. There is, however, a tendency toward individual variability in the relative importance of each. For example, L. S. stood second highest largely by virtue of high efficiency while J. R., who had greatest total output, owed his predominance clearly to a high capacity to consume oxygen (C.L.O.C.).

SUMMARY AND CONCLUSIONS

Section I. 1. Heart rates in the steady state of exercise of 45 minutes' duration reach a level depending on the intensity of the work but continue to rise slightly throughout the experiment. They do not, in the typical case, maintain an absolutely steady state. Equations for this slope are given for two subjects in 24 experiments each.

2. Significantly high correlations (0.969 ± 0.013 and 0.955 ± 0.020) were found between heart rate and work-load for the two subjects.

3. Partial regression equations express the influence of training in lowering the heart rate during work in the two subjects.

Section II. 1. Circulatory and respiratory responses of three male and two female subjects were studied in work experiments lasting 30 minutes.

2. All functions except heart rate tend to reach a plateau at not later than ten minutes of exercising time and fluctuate somewhat about a horizontal curve. Heart rate, as shown above, slowly rises throughout the experiment.

3. Equations are given for the regressions of heart rate, systolic pressure, diastolic pressure, pulse pressure and total ventilation on work-load in the cases of the three male subjects. Curvilinear relations in these functions precluded the calculation of these values for the two female subjects.

4. A comparison of the responses of male and female on an equal weight basis shows that significant differences occur only in heart rate and respiratory rate which are higher in the latter.

Section III. 1. The circulatory, respiratory and metabolic responses of a group of subjects to a periodically increasing work-load on a bicycle ergometer were studied. All experiments were continued uninterruptedly until the subject was forced to quit from exhaustion.

2. Heart rate, total ventilation, oxygen consumption, and respiratory quotient increase with work-load in approximately linear fashion. The slope of the regression line and the ultimate level reached are individual variables.

3. The behavior of these functions at the approach of exhaustion is determined by comparing trends throughout the range of work-loads to the trend at the uppermost step at which exhaustion occurs. The exhaustion trends of metabolic and circulatory functions are lacking in uniformity. Total ventilation, on the other hand, most often becomes markedly excessive.

4. The alveolar pCO_2 and per cent CO_2 in expired air both increase in the transition from rest to work, remain on a fluctuating plateau throughout most of the work range and decline sharply at exhaustion. These variables as well as ventilation most reliably signal the onset of exhaustion in an objective fashion.

5. The rôles of efficiency and magnitude of oxygen consumption at the crest-load in determining total work capacity have been shown graphically.

6. The step-up experiment offers promise in the measurement of the important physiological factors underlying individual physical capacity for sustained heavy exercise.

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OBSERVATIONS ON THE RETURN OF VASCULAR TONE AFTER SYMPATHECTOMY

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"Vascular tone" is a rather indefinite but convenient term applied to the state of partial contraction in which blood vessels, especially arteries, are usually found. (For general literature, see Cannon, 1937; Heymans and Brouha, 1937; McDowall, 1938; and Hermann, 1939.)

Because this more or less contracted condition is markedly influenced by the nervous system, many attempts have been made to study it in vessels which have been disconnected from nervous control. Among the most painstaking observations on the behavior of denervated blood vessels were those of Grant (1930, 1935), and Grant and Bland (1932). After careful experiments on the normal and the denervated rabbit's ear, he concluded (1935) that the regain of tone after denervation is probably due to an increased responsiveness of the denervated vessels to various stimuli, including an adrenaline-like substance circulating in the blood stream. Grant argued that this substance came from neither the adrenals nor the pituitary body and that it was unlikely to be sympathin. He found that its concentration in the blood was increased by nervous or muscular activity and reduced by rest.

Since the return of vascular "tone" after sympathectomy is usually attributed to an "inherent" or "intrinsic" property of the vessel wall, it seemed desirable to follow up the stimulating suggestion of Grant. Accordingly, two series of experiments were carried out, one on the normal and denervated ears of albino rabbits, the other on sympathectomized cats.

I. *Observations on the Rabbit's Ear. A. Methods.* Young albino rabbits of both sexes were used, 23 in all. Most of them weighed between 1.7 and 2.4 kgm. The ear was denervated by *a*, removal of the superior cervical and stellate ganglia on one side, or *b*, removal of the superior cervical ganglion and cutting of the dorsal auricular and great auricular nerves at the base of the ear on the same side. No definite difference was found in the reactions of the vessels or ears deprived of their sympathetic

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supply by either the first or the second operation.[2] Method *b* was used in the majority of instances, since removal of the stellate ganglion in the rabbit is rather difficult. Only one ear was denervated in each animal, the other being used as a control.

The ear vessels were observed as the animal rested quietly, usually on an electric warming pad. It was possible to lay the ear gently on an ordinary microscope stage and measure changes in caliber of the vessels with a micrometer eye-piece and low-power objective. Before a rabbit was operated upon its ear was observed on one or more days and mapped to show the location of the major arteries. On this map were indicated the segments of vessels on which measurements were made. Thus measurements could be made both before and after denervation on exactly the same part of the same artery.

Occasionally the direct measurements of vessel diameter were supplemented by temperature recordings taken by means of iron-constantan thermocouples attached to the ears with adhesive tape and connected to a Leeds and Northrup "Micromax" Recorder. Adrenaline and other drugs were injected into a leg vein (to avoid manipulation of the ears), usually after the animal had been rendered drowsy by intra-peritoneal injection of nembutal (29 mgm. per kgm. in most instances). Blood pressure was taken in some animals by use of a celluloid ear capsule and rubber diaphragm similar to the apparatus described by Grant and Rothschild (1934).

B. *Results.* 1. *The effect of body temperature.* Grant's (1935) thermocouple records showed that if a rabbit with one sympathectomized ear is gradually chilled the normally innervated ear vessels begin to constrict when a certain crucial level of rectal temperature is passed, while the denervated vessels remain dilated. A similar experience is recorded in figure 1. A rabbit, with right ear denervated 6 days previously, was placed outside a window in a box on a cold day for one hour. Its rectal temperature was then 37.0°C. The animal was thereupon warmed slowly on an electric warming pad. As its temperature rose, an artery of the normal ear, at first constricted, gradually dilated, as shown by direct measurements of its diameter. The denervated artery, however, was dilated at the start, and remained so, independent of the change in body temperature. Several similar experiments confirmed the conclusion of Grant, that there exists in the rabbit a certain threshold level of body temperature (about 38.5°C.), above which the normally innervated vessels

[2] Grant (1932) has shown that, in order to denervate the *entire* ear, not only the two nerves mentioned above must be cut but also the auriculotemporal branch of the trigeminal nerve and the auricular branch of the vagus. The distal third of the ear, however, may be completely denervated by cutting the dorsal and great auricular nerves and removing the superior cervical ganglion (see also White, Okelberry and Whitelaw, 1936). In the present series observations were made on the distal third of the ear.

are dilated, below which they are constricted, while the denervated vessels remain dilated (at least over a range of several degrees). It follows that, when the vascular reactions of the ears are being studied, the rabbit's temperature should be above this critical level, in order to provide a background of vessels in the fully dilated state. Otherwise the extent of constriction in response to a given stimulus cannot be gauged.

2. *The pattern of reactivity in normal and denervated vessels.* It was found (again confirming Grant) that excitement, and particularly struggle, induced by simply holding one hind leg for a few seconds, constituted an

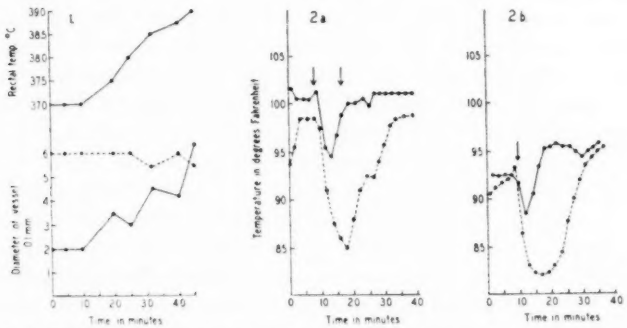

Fig. 1. Rabbit 1. Right ear denervated 6 days previously. Graphic record of experiment in which the animal was chilled until its rectal temperature was 37°C., then gradually warmed over some 40 min. Rectal temperature is recorded above, diameter of comparable vessels of the two ears below. Note that the normally innervated vessel (solid line), at first constricted, dilated as the body temperature rose, while the denervated vessel (dotted line) remained constantly dilated.

Fig. 2a. Rabbit 2. Temperature records from normal ear (solid line) and denervated ear (dotted line) showing the different response of the two ears to the same stimulus (struggle) applied during the time indicated by the two arrows. Compare with figure 2b.

2b. Rabbit 2. Temperature records from normal (solid line) and denervated ear (dotted line) showing response to single intravenous injection of adrenaline (at point marked by arrow). An intraperitoneal injection of nembutal (35 mgm. per kgm.) was given about 20 min. before the record was started. Compare with figure 2a.

effective stimulus to constriction, for both normal and denervated vessels. If the quiet, warm animal, with both ears fully flushed,[3] is caused to struggle, the normal ear quickly pales; within a few seconds its main arteries become much smaller in caliber, only to relax with comparable rapidity. The

[3] Grant (1935) points out that while, in the warm and resting animal, the main vessels and their small branches are fully relaxed, the minute vessels (i.e., capillaries), though dilated, are not maximally so. This fact can be demonstrated by the direct application of heat to the ear, with a resulting slight deepening in the "ground tone" or background color of the ear. No change occurs, however, in the size of the visible vessels.

vessels of the denervated ear, on the other hand, exhibit a much more gradual constriction, and the return to the complete dilation of the resting state may require several minutes.

This type of observation, which was made repeatedly, is illustrated in figure 2a. Here are represented temperature records from a normal and a denervated ear in the same rabbit. It will be noted that at first the denervated ear was relatively cool, as it always is in the excited or disturbed rabbit. After a few minutes of rest on the warming pad the vessels relaxed. Struggle was then instituted by subjecting the animal to restraint, and continued for several minutes. The left (normal) ear became rapidly cooler, then began to turn warm again even before the struggle had ended. The denervated ear, on the other hand, became cooler more gradually and more slowly, and, after the struggle had ceased, required several minutes to regain its original temperature. This response of denervated vessels to struggle is typical and could be reproduced at will in both rabbits and cats (see fig. 4a). The temperature changes recorded were, of course, paralleled by alterations in the caliber of the vessels as seen directly. (It should be pointed out that the temperature recorder, printing once a minute, could not follow the state of the vessels at every instant. Also, the temperature of the ears cannot change as rapidly as the diameter of the vessels. For example, in figure 2a, the vessels of the left or normal ear actually constricted almost to invisibility, but relaxed again so rapidly that the recorded temperature did not fall below 94.5°F.)

The vascular reaction represented in figure 2a is to be compared with that shown in figure 2b, from the same rabbit. Here an intraperitoneal injection of nembutal (35 mgm. per kgm.) was given about 20 minutes before the record was started, a necessity because the manipulation attending intravenous injections produces enough excitement to cause constriction of the ear vessels. At the time indicated, when the rabbit was under moderately deep narcosis, adrenaline was injected intravenously in a dose found previously to produce a maximal effect on the denervated ear and only a transitory paling of the ground color of the normal ear. The time courses of changes in the two ears are much alike in 2a and 2b.

3. *The return of "vascular tone."* Goltz (1874) noted that the warming effect on the dog's leg of section of the sciatic nerve did not persist; indeed, that the operated leg might even become cooler than its fellow. Dale and Richards (1918) recorded similar observations in the cat. They noted increased sensitivity of vessels of the denervated limb to adrenaline, acetylcholine and histamine, and suggested that the reactivity of normally innervated vessels is restricted by the effect of tonic impulses from the nerve centers. According to Grant (1935) the tendency to constriction of the denervated vessels of the rabbit ear increased for about 5 to 7 days, after which no further change occurred.

In the present investigation constriction of the vessels of the denervated

ear was usually most marked in animals operated on a week or more previously, and least marked during the first two or three days after operation. When the reaction was well-developed a difference between the two ears was often perceptible immediately after the animal was brought from the animal room to the laboratory. Ordinarily, if the animal was kept warm and quiet, the constricted vessels would relax within several minutes and the two ears would become flushed and indistinguishable one from the other. The initial constricted state of the denervated vessels, however,

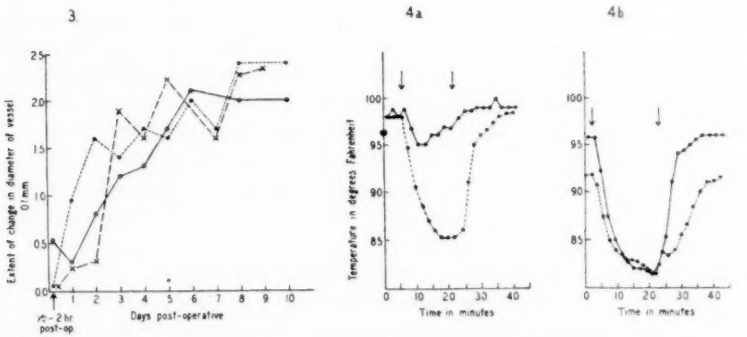

Fig. 3. Graphs of the increasing degree of constriction of the rabbit's ear vessels to various stimuli on successive days after being denervated. Each point on the graphs marks the average of observations on several rabbits on the same post-operative day. The solid line represents the extent of constriction, while the animal is in a resting state, as compared with the diameter of the same vessel before operation, i.e., "tone." The dotted line expresses the extent of constriction (i.e., change in diameter) in response to struggle. The dash-line shows the extent of constriction in response to a standard dose of adrenaline.

Fig. 4a. Cat 1. Sympathectomized (see text). Effect of struggle on ear temperature 10 days after removal of superior cervical ganglion and section of peripheral nerves to *right* ear. Solid line: innervated ear. Dotted line: denervated ear. Struggle began at the time indicated by the first arrow, and the animal was released at the second arrow.

4b. Same cat as figure 4a. Record obtained 4 days after removal of *left* superior cervical ganglion. Solid line: left ear. Dotted line: right ear. Struggle began at first arrow, ceased at second arrow.

could be used as a crudely quantitative measure of vascular "tone." This became more evident when measurements on a series of rabbits were combined and plotted (see fig. 3, solid line). Each point in the figure represents the average of observations on several rabbits on the same day after operation. The ordinates represent the measured difference, in tenths of a millimeter, between the diameter of the vessel before operation and the diameter of the same part of the same vessel on the post-operative day indicated by the abscissae. For instance, if the diameter of an artery was 0.7 mm. before operation and 0.5 mm. when observed on the third day,

the difference (0.2 mm.) was recorded and averaged with other observations made on the same post-operative day.

Similarly it was possible to record, in a roughly quantitative way, the responses of the vessels to struggle (of approximately 30 seconds' duration) and to a standard dose of adrenaline (0.002 mgm.) given intravenously. Struggle was induced only when the animal was in a warm, resting state, with the vessels of both ears well dilated. The diameter of the vessel, measured at the time of maximal constriction, was subtracted from the diameter recorded before the struggle began. An average of several such measurements gave a point on the graph. For the adrenaline tests it was necessary to quiet the animal with nembutal (given intraperitoneally). The diameter of the vessel as then recorded was compared with the diameter when maximally constricted by adrenaline. The dose of adrenaline selected was one which produced in the *normal* ear only some paling of the ground color, without change in the diameter of the main or visible vessels. It should be noted that all observations summarized in figure 3 were made on arteries of comparable size, i.e., 0.6 to 0.8 mm. in diameter in the dilated state.

In one rabbit, 28 days after denervation of the right ear, both adrenal glands were removed. The animal was kept alive for 5 days with the aid of subcutaneous injections of saline solution. The vessels of the denervated ear tended to remain in a moderately constricted state, even when the animal was warm; this response is typical of animals which are sick or recovering from operation. On two occasions, however, relaxation of the vessels was secured; struggle then produced the typical response (cf. fig. 2a). Also, the increased sensitivity of the denervated vessels to injected adrenaline was unchanged.

II. *Observations on Sympathectomized Cats.* A. *Methods.* Cats, usually large males, were sympathectomized by a modification of the method of Cannon *et al.* (1929). Usually the operation was done in two stages, in order to preclude regeneration as far as possible (see Discussion). The first stage consisted in removing the thoracic sympathetic chain on one side (cutting its splanchnic nerves) and removing both abdominal chains. The second stage, carried out as soon as the animal recovered its appetite (usually in about 10 days), involved removal of the remaining thoracic chain with section of its splanchnic nerves. Thus the sympathetic chain on one side was removed *in toto* at the first stage, while the other was removed in two pieces. In this way several animals were made ready for study within 2 or 3 weeks after the *first* stage; one cat was used 10 days after the first stage of the operation. Several other animals were used which had been sympathectomized for longer periods.[4]

[4] The author is indebted to Dr. Robert Hodes for permitting him to denervate the ears of and make observations on cats which had been sympathectomized for another purpose.

Denervation of an ear was carried out by removing the superior cervical ganglion on one side, then making an incision over the parotid gland and cutting the following nerves at the base of the ear: great auricular, great occipital, auricular branch of the auriculo-temporal, and auricular branch of the vagus (the posterior auricular branch of the facial nerve was cut along with the auricular branch of the vagus, since it was impossible to separate the two). It should be noted that complete vasomotor denervation of the ear, in either the cat or rabbit, is secured only by cutting the peripheral sensory nerves; some of these nerves contain antidromic dilator fibers, as shown by electrical stimulation of the distal end of the cut great auricular nerve in a chronically *sympathectomized* ear, whereupon the ear immediately becomes flushed and warm. (That these vasodilators are activated reflexly, however, is not clear; see Dole and Morison, 1940.) Also, to produce complete *sympathetic* denervation alone, it is necessary to remove the stellate ganglion besides the superior cervical, since vasomotor fibers to the ear leave the stellate ganglion by way of its vertebral ramus.

Denervation of one ear was usually done first; then the two stages of the sympathectomy were completed, the second as soon as possible after the first. The animals were usually ready for observations within 5 or 6 days after the last stage. The time between the *first* stage of sympathectomy and the first record of ear temperature on the totally sympathectomized animal was as follows: less than 15 days, 4 cats; 15 to 23 days, 5 cats; 7 weeks, 1 cat; 2 months, 2 cats.

The reactions of the two ears were studied in the *unanesthetized* animal by direct observation and by recording temperature on the Micromax machine. Usually, the animal was allowed to rest quietly on an electric warming pad, with thongs placed about its legs; after the record had been started, the thongs were suddenly fastened to the cleats of the animal board, thus causing the cat to become quickly excited and to struggle. After this activity had continued for some minutes, the animal was released. As a rule it again rested quietly on the warming pad, and was encouraged to do so by stroking its back. When the temperature of the two ears had returned to approximately the original levels, the record was stopped.

When several such records had been taken on different days, the animal was sacrificed in an acute experiment. Anesthesia was, in most cases, urethane (about 1.0 gram per kgm. intravenously); in 2 animals, ether (by inhalation through a tracheal cannula from a bottle with adjustable outlets) was used; and in a few, dial (Ciba, 0.7 cc. per kgm. intraperitoneally). A blood-pressure cannula attached to a Hürthle manometer was inserted in most cases into a femoral artery; in a few instances where the cannula was placed in a carotid artery no interference with the vascular reactions of the ears was detected. Drugs were injected into a femoral

vein. Stimulation of nerves, to test vascular reflexes,[5] was carried out by means of shielded electrodes attached to a Harvard inductorium with 5 volts in the primary circuit; the secondary coil was set usually at 6 or 8 cm.

B. *Results.* 1. *Evidence for a humoral factor.* Twelve cats were successfully sympathectomized and had one ear denervated as described above. Of these, 10 showed a clear-cut response of the type shown in figure 4a. It will be noted that the denervated ear became much cooler than the other and that the general shape of the curve is similar to that obtained in rabbits (fig. 2a). The denervated ear, even in the animal without sympathetic innervation, seemed more sensitive than the other to humoral stimuli. The different sensitivity of the two ears is explicable in the well-known difference, in sensitivity to adrenaline, resulting from pre- and postganglionic denervation (Hampel, 1935). This inference is confirmed by the behavior of the ear temperature in two sympathectomized cats in which *both* superior cervical ganglia were removed. The temperatures of the two ears followed similar courses (cf. fig. 4a and 4b). Similarly, in cat 2, after removal of both superior cervical ganglia, the temperatures of the two ears fell and rose together, when struggle occurred, to about the same extent as the unilaterally sensitized ear. Additional evidence for the greater sensitivity to humoral stimuli of the vessels of the denervated ear is given by the effect of adrenaline (fig. 5).

2. *Effect of drugs.* From the foregoing evidence it seemed probable that, even in the totally sympathectomized animal, some humoral agent was causing the marked fall in temperature of the denervated ear when struggle occurred. Several substances which might have been at work were, therefore, injected into the anesthetized animals in the final, acute experiments. In figure 5 are illustrated the effects of intravenous injection of adrenaline, pitressin and acetylcholine on the blood pressure and ear temperatures of a sympathectomized cat with one ear wholly denervated. Adrenaline was the only one of the three drugs which mimicked the effect of struggle on ear temperature.[6] Indeed, although adrenaline and pitressin produced similar elevations of blood pressure (though of different duration), their effects on the ears were widely different, that of pitressin being actually to warm the non-denervated ear. This distinction was confirmed several times in different animals. The indefinite effect of acetylcholine on the ear was also obtained repeatedly. The effect of histamine (not illustrated) was also negligible on repeated trials and in different doses.

[5] Some of the observations on vascular reflexes were carried out in association with Dr. Robert Hodes.

[6] The situation is complicated by the fact that a *fall* of blood pressure occurs in the unanesthetized, sympathectomized cat during struggle. This point is taken up in the Discussion.

An attempt was made to see if the action of the hypothetical adrenaline-like substance could be potentiated by cocaine (cf. Rosenblueth and Schlossberg, 1931). Cat 4 was given 5 mgm. of cocaine per kgm. 40 minutes before struggle was started. The denervated ear became several degrees colder than it did on other occasions during struggle without preliminary injection of cocaine.

3. *Source of adrenaline-like substance.* It seemed likely that a humoral substance resembling adrenaline was producing the cooling of the de-

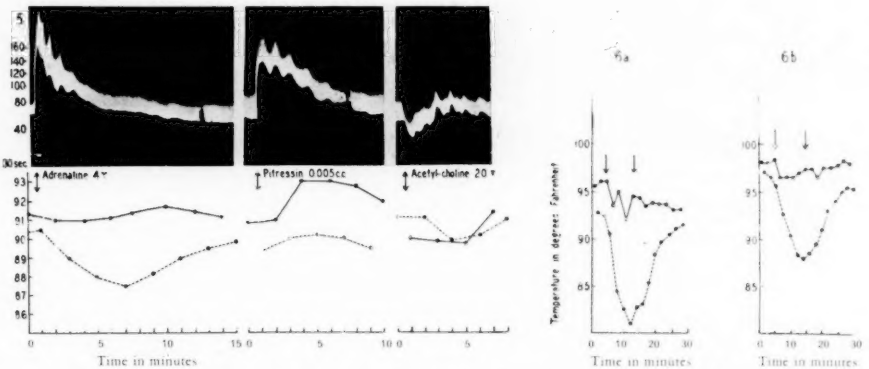

Fig. 5. Cat 3. Sympathectomized (see text). Simultaneous records of blood pressure and ear temperature to show the effect of drugs. The adrenaline and pitressin were Parke-Davis preparations, the acetylcholine Merck's, in freshly prepared solution. The drugs were injected at the time indicated by the signal marker. The solid line represents the innervated ear, the broken line the denervated ear.

Fig. 6a. Cat 5. First stage of sympathectomy 23 days previously. Second stage and denervation of ear 10 days before. Struggle took place in the period between the two arrows. Solid line: innervated ear. Broken line: denervated ear.

6b. Same cat as figure 6a. Three days after bilateral adrenalectomy. Response to struggle. Lines represent conditions as in figure 6a.

nervated ear during struggle. The most obvious source for such a substance was the adrenal glands themselves, although the operation of total sympathectomy should have inactivated them.

With this possibility in mind, the adrenal glands were completely removed in one of the sympathectomized cats, as shown in the following protocol:

Cat 5.

3/ 1/40 First stage sympathectomy (left thoracic chain (with left splanchnics) and both abdominal chains removed).

3/13/40 Second stage. Right thoracic chain removed (with right splanchnics), right ear denervated by usual method.

3/15/40 Animal apathetic. Thermocouple record shows right ear several degrees warmer than left. No struggle.

3/23/40 Thermocouple record during struggle. Temperature of right ear fell more than 10°F. (Fig. 6a.)

3/26/40 Both adrenal glands removed. Abdomen filled with saline solution before closing. Intramuscular injection of 1 cc. adrenal cortical extract (Wilson) given and repeated daily thereafter.

3/29/40 Temperature record during struggle (fig. 6b). Curve similar to that obtained before.

4/ 9/40 Sacrificed in acute experiment. Records not significant because of extremely low blood pressure. Autopsy showed a single sympathetic ganglion under the diaphragm on the right side. No accessory adrenal tissue discovered.

Thus it became clear that the presence of the adrenal glands was not essential for the typical response of the denervated ear to struggle. However, the finding at autopsy of a single sympathetic ganglion, apparently missed at operation, was of considerable interest, for it suggested that sympathin, even though in extremely minute amounts, might be the active agent. Newton, Zwemer and Cannon (1931) noted that an acceleration of the denervated heart, which occurred in an animal presumably quite sympathectomized, disappeared when small remnants of the lower thoracic sympathetic chains were removed. Careful autopsies, in the present series, revealed no evidence of incomplete sympathectomy in any of the cats other than the one just mentioned. It is admittedly difficult, however, to find missed ganglia at autopsy, especially when much scar tissue is present.

The possibility was considered that sympathin might be produced by activity of decentralized ganglia, e.g., the celiac or mesenteric (Govaerts, 1935). In order to test this hypothesis, on 6/13/40 the celiac, superior and inferior mesenteric ganglia were removed in cat 6, which had had a first stage sympathectomy on 5/17/40, denervation of the left ear on 5/23/40 and second stage sympathectomy on 5/29/40. Figure 7a shows the thermocouple record during struggle on 6/11/40, while figure 7b shows the record obtained on 6/17/40, 4 days after removal of the celiac and mesenteric ganglia. Again there is no significant difference.

Cannon and Bacq (1931) showed that the rate of the denervated heart is a sensitive indicator of the presence of circulating sympathin. Satisfactory records of the heart rate were obtained in acute experiments on 5 of the sympathectomized cats, after section of both vagi. In 4 of the 5 the heart rate was constant within 4 beats per minute during stimulation of the central end of the depressor nerve or a sensory nerve or both. Of these 4 animals, 3 showed the typical temperature effect on the denervated ear from struggle, and one did not (see below).

Considerable interest attaches to the behavior of 2 cats (nos. 7 and 8), out of the series of 12, which failed to show the typical cooling effect of

struggle on the denervated ear. One was first used 15 days, the other 21 days, after the *first* stage of the sympathectomy. As shown in figure 8, each exhibited a questionable mild response involving both ears to about the same extent. A striking feature of the general behavior of these two animals was their tendency to faint on the slightest provocation. This tendency of freshly sympathectomized cats to faint when held vertically with the head up or when exercised is well recognized (cf. Hodes, 1940). The mechanism of this phenomenon is not clear; it is, however, accompanied by a marked slowing of the heart rate, and may be fatal if the animal is not at once placed in a head-down or horizontal position. Such fainting was observed in many of the cats, but was most marked in the

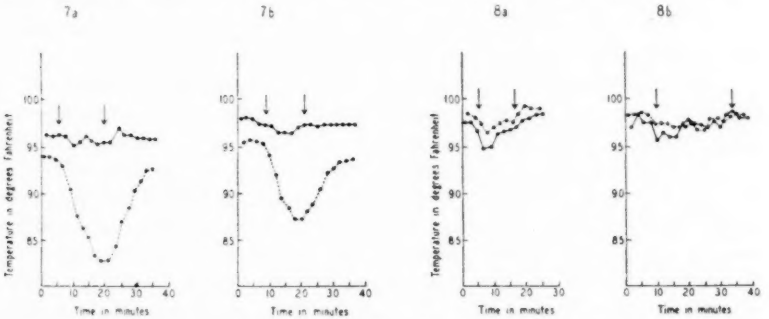

Fig. 7a. Cat 6. Sympathectomized. Record of ear temperatures 24 days after first stage of sympathectomy. Lines represent conditions as in figure 6a.

7b. Cat 6. Response of ear temperatures to struggle 4 days after removal of celiac and mesenteric ganglia. Lines represent conditions as in figure 6a.

Fig. 8a. Cat 7. Sympathectomized. Record of ear temperatures showing absence of usual response to struggle. Lines represent conditions as in figure 6a.

8b. Cat 8. Sympathectomized. Record of ear temperatures showing absence of usual effect of struggle. Lines represent conditions as in figure 6a.

two just mentioned; for instance, cat 7 fainted while being given ether at the outset of the acute experiment, and cat 8 fainted while resting fairly quietly in an animal box and having its ears shaved. Furthermore, the depressor reflex, elicited by electrical stimulation of the central end of the vagus or sciatic nerve, which was quite clear-cut in several of the animals, was seen in these two only when stimulation was strong enough to evoke extensive muscular movement.

Discussion. It is apparent, from the experiments described above, that the denervated ear is an exceedingly sensitive indicator of some circulating vasoconstrictor substances. The results give support to the thesis of Grant, that the return of "tone" in denervated blood vessels is best regarded as a return of *reactivity*, i.e., an increasing sensitiveness to

and responsiveness to humoral stimuli. This conclusion is supported by the following facts:

a. In both the rabbit and the sympathectomized cat, if certain factors such as body temperature and activity were controlled, there was no evidence of return of a constricted state in the vessels of the denervated ear, aside from what was most reasonably interpreted as response to a circulating vasoconstrictor substance.

b. In the rabbit the return of "tone," i.e., a constricted state of the vessels in the resting animal, was shown to develop *pari passu* with sensitivity to adrenaline and to whatever vasoconstrictor substance or substances appear in the blood during struggle.

c. In both the rabbit and the cat adrenaline was the only drug, of those tested, that fully mimicked the effect of struggle. Pitressin, histamine and acetylcholine produced different results.

d. In an adrenalectomized rabbit, and in an adrenalectomized cat in which a single sympathetic ganglion remained, the ear response was the same as before removal of the adrenals.

e. In two of the sympathectomized cats the typical constrictor effect in the ear was not obtained. These two animals were both freshly sympathectomized, both showed a tendency to faint readily, and neither showed a definite depressor reflex. The absence of fall of temperature of the denervated ear in these animals appears to rule out a drop of blood pressure as the cause of the differential effect. Furthermore, this effect appeared in rabbits in which the blood pressure rose during struggle.

The humoral agent to which the denervated vessels were responding in the above experiments is apparently an adrenaline-like substance, but not adrenaline itself, certainly not in all cases. One might conclude, with Grant, that it is a hitherto unknown vasoconstrictor. Even in the absence of conclusive evidence, however, it may be sympathin. One must then assume that it was present in the sympathectomized cats either as a result of incomplete operation or of regeneration.[7] And this assumption must be made although several of the cats had a constant heart rate during sensory nerve stimulation and although in only one sympathectomized cat was any anatomical evidence of incomplete operation or of regeneration encountered.

[7] One other possible source of sympathin is suggested by the demonstration that vagal cardio-accelerator fibers may be excited by exercise and by cerebral ischemia (Hodes, 1940; Kabat, 1940) and that the effects of such excitation are probably mediated by sympathin. It may be questioned, however, whether sufficient sympathin to cause constriction of the ear vessels could be produced in this way. As will be seen below, struggle and cerebral ischemia in the sympathectomized cat, without atropine, often produce slowing of the heart, and in the present experiments the typical constrictor response of the ear vessels was often seen in association with slowing of the heart.

If circulating sympathin is the vasoconstrictor agent, the importance of complete operation and prompt use of animals in an experiment involving sympathectomized animals is obvious, especially since regeneration is said to occur as early as 3 weeks after operation (for literature see Haimovici and Hodes, 1940). Indeed, the presence of a circulating vasoconstrictor substance may account, in some instances, for the discordant results obtained by those who have studied blood-pressure reflexes in sympathectomized animals (Freeman and Rosenblueth, 1931; Bacq, Brouha and Heymans, 1934; Rosenblueth and Cannon, 1934; Pinkston, Partington and Rosenblueth, 1936; Thomas and Brooks, 1937; Bacq, Bremer, Brouha and Heymans, 1939; Brown and Maycock, 1940). It is noteworthy that most of these investigators report only the time elapsing from the *last* stage of sympathectomy until the animals were used, whereas opportunity for regeneration was present, obviously, from the time of the *first* stage.

The hypothesis outlined above admittedly cannot be used to explain *all* vascular changes observable in the sympathectomized cat. For instance, the recovery of blood pressure after the first stage of the operation may be assumed to be due in part to increased sensitivity of the denervated vessels to circulating vasoconstrictor substances; but continued recovery after the second stage, if the operation is complete, may well be due partly to increased blood volume (cf. Cannon, 1937), some of the increase having probably occurred between the first and second stages.

Another obscure aspect of the behavior of sympathectomized cats, mentioned above, is fainting, associated with fall in blood pressure, which often occurs when such animals struggle (Freeman and Rosenblueth, 1931). The slowing of the heart under such conditions has been attributed to cerebral ischemia with resultant excitation of the vagal centers (Hodes, 1940). The cause of the fall of blood pressure, however, remains unclear. It has been attributed to activation of dorsal root dilators (Rosenblueth and Cannon, 1934) and to vasodilatation due to muscle metabolites (Bacq, Brouha and Heymans, 1934). The possibility that acetylcholine liberated during struggle (Bender, 1938a, b) is in some way involved, must be considered. As noted above, in the present experiments no noteworthy evidence was found of dilatation of the ear vessels during the fall of blood pressure induced by intravenous injection of acetylcholine.

The experiments recorded above appear to give no support to the concept of "myogenic" or "intrinsic" tone, in the sense in which the term is often used to imply the existence of a mysterious tendency to contraction on the part of the smooth muscle of a denervated artery. The evidence for the existence of such a property of the arterial wall is usually drawn from observations on denervated vessels in an otherwise intact animal, or from the appearance of rhythmic contractions in bits of excised artery kept in warm Ringer's solution. According to the results described above, the de-

nervated artery in an otherwise intact animal is sensitive to, and often responding to, circulating vasoconstrictor substances. If these humoral stimuli are eliminated, it is difficult to find convincing evidence of "tone" in the denervated vessels. The data on excised segments of artery are open to criticism, since it is doubtful whether extraneous physico-chemical stimuli can be said to be eliminated when tissue is handled in such a traumatic and unphysiological manner.

SUMMARY

Conditions were studied that induced vasoconstriction in the denervated ear vessels of rabbits and sympathectomized cats.

The diameter of denervated vessels (rabbit), unlike those normally innervated, is not affected by moderate changes of body temperature (fig. 1).

Struggle induces constriction (cooling) of denervated vessels (rabbit), similar to the constriction caused by adrenaline (fig. 2a and b), an effect more marked as days pass after the operation (fig. 3).

In the sympathectomized cat struggle produces constriction (cooling) of the denervated ear (fig. 4a and b).

Of the three drugs—acetylcholine, pitressin and adrenaline—only the last has effects resembling the effects of struggle (fig. 5).

Removal of the adrenal glands from a sympathectomized cat does not exclude vasoconstriction in the denervated ear (fig. 6a and b); nor does removal of prevertebral ganglia (fig. 7a and b).

Absence of the usual auricular vasoconstrictor effects of struggle may occur in sympathectomized cats (fig. 8a and b).

No evidence was obtained for the existence of "myogenic" or "intrinsic" tone; it can best be explained as a response of the sensitized smooth muscle of the blood-vessel wall to circulating vasoconstrictor substances.

The experimental results are therefore regarded as supporting the thesis of Grant (1935) that the return of "tone" in sympathectomized blood vessels is due to a return of reactivity, i.e., a heightened responsiveness to humoral stimuli.

It is concluded that, under the conditions of the experiments, the vasoconstrictor substances concerned were adrenaline and sympathin.

The denervated ear is an exceedingly sensitive indicator of the presence of circulating vasoconstrictor substances. The importance of using sympathectomized animals promptly to avoid confusing regeneration is stressed.

The author wishes to express his thanks to Dr. W. B. Cannon for his helpful advice and interest in the problem.

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THE ADRENAL GLAND AND FOOD INTAKE[1]

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Work with adrenalectomized ground squirrels (Citellus tridecemlineatus) indicated that the food intake of these animals dwindles after the end of a short post-operative recuperative period. Body weight change is dependent upon food intake change. Desire for food can be maintained or restored by providing the animals with 1 per cent sodium chloride solution to drink. Survival depends upon the maintenance of food intake. Fasted adrenalectomized ground squirrels drinking 1 per cent sodium chloride solution do not survive any longer than do fasted adrenalectomized ground squirrels drinking water; but blank (sham, mock) adrenalectomized animals can survive a fast at least four days longer than the longest endured by adrenalectomized ground squirrels.

This paper is a report of more detailed experiments on albino rats which were designed to show:

1. The effect upon food intake accompanying untreated adrenal insufficiency.

2. The effect of sodium chloride treatment upon the food intake of adrenal insufficient animals.

3. The difference between the desire for food of untreated and of sodium chloride treated adrenalectomized animals.

4. The effect of a food deprivation such as that undergone by untreated adrenalectomized animals upon blank adrenalectomized animals and upon adrenalectomized salt treated animals.

METHODS. Approximately the same number of male and female rats were used in each experiment. They were kept in the laboratory about the same length of time before operation. They received a diet of yellow corn ground fine, wheat middlings and casein, with added mineral and vitamin supplements.

The animals were anesthetized by Nembutal injected intraperitoneally and usually followed by ether. Operations were performed under moderately aseptic conditions. In adrenalectomy, both glands were removed at one operation by a dorsal approach. Blank adrenalectomy consisted of making the incisions and handling or putting stress on the adrenals and

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usually the kidneys, liver, spleen and pancreas. All animals were allowed to recover from the operation for three days before being subjected to further experimentation. The adrenalectomized rats were given 1 per cent sodium chloride solution to drink during this period, and all recovered well.

The animals were kept singly in wire-bottom cages. Food and drinking fluid were offered in sponge cups. Fluid consumption was measured daily in most cases. Food consumption and weight were recorded in all cases either daily or every third day. In cases where sodium chloride solution was injected subcutaneously, a sterile solution was employed.

RESULTS. 1. *The drop in food intake of untreated adrenalectomized rats, and the effect of sodium chloride treatment upon the food intake of adrenalectomized rats.* Twenty-four adrenalectomized rats, including castrates of both sexes, were allowed free access to water and food after the three day recovery period. All died. The average survival after the recovery period was 15.1 days (range 6.8–25.5). The older, heavier animals survived the longest. The average of the weight at death in percent of the weight at the end of the recovery period was 75.8 (range, 64.5–87.2). Twelve adrenalectomized rats, ten weeks old at operation, were allowed free access to one per cent sodium chloride solution and food. All survived. Twenty-one days after operation the average weight in per cent of the weight at operation was 112.1 (range, 102.8–123.2).

Table 1 gives the weight and food intake changes of typical male and female members of each of the above two groups.

2. *The immediate effect on the appetite of adrenalectomized rats of withdrawal and subsequent restoration of 1 per cent sodium chloride solution to drink.* Three adrenalectomized males and four adrenalectomized females which had been maintained on 1 per cent sodium chloride solution were used. The procedure was to substitute water for 1 per cent sodium chloride solution as the drinking fluid, and then to replace the water by 1 per cent sodium chloride solution when the animal appeared to be in danger of succumbing to adrenal insufficiency. Three of the animals were so treated on three different occasions and one on two different occasions. The tests were made 6 to 17 weeks after adrenalectomy. In the 14 cases of substitution of water for 1 per cent sodium chloride solution, the average weight of the animals three days after the change, in per cent of their weight at the time of the change, was 90.2 (range 86.0–94.3). In 8 of the 14 cases the food intake was recorded. The average of the average daily food intake for three days after the change, in per cent of the average daily intake for three days before the change, was 44.9 (range 33.3–59.3). These changes in appetite are only slowly progressive. The first day changes are of about the same magnitude as the average first three day changes. Even the most extreme normal three day fluctuations in food intake are small in comparison to these figures. Immediate measurements were not made in most

cases after replacement of water by one per cent sodium chloride solution, but it was simply observed that the animal in each case began immediately to drink, drank a large quantity within an hour or two, and thereafter exhibited a normal appetite. In six cases in which the animal was weighed one hour after the replacement of water by 1 per cent sodium chloride solution, the average weight gain was 25.3 grams (range 20.5–30.5) most of

TABLE 1

Weight change and food intake of adrenalectomized rats drinking water and of adrenalectomized rats drinking 1 per cent NaCl solution

		WEIGHT 6TH DAY BEFORE OPERATION	WEIGHT AT OPERATION	AVERAGE FOOD CONSUMED DAILY, 6 DAYS BEFORE OPERATION		DAYS AFTER OPERATION						
						3	6	9	12	15	18	21
		grams	*grams*	*grams*								
♂	33*	223	246	20	Weight‡	101.4	94.3	88.9	82.1	76.4	74.4	65.4**
					Feed§	77.5	50.0	25.0	12.5	12.5	17.5	3.3**
♂	42†	255	279.5	22	Weight	102.2	103.1	103.4	103.9	107.2	109.8	111.5
					Feed	70.4	90.9	84.1	93.2	93.2	95.4	106.8
♀	16*	162.5	172	13.5	Weight	102.2	97.4	86.9	81.4	75.6††		
					Feed	81.5	77.8	29.6	25.9	9.6††		
♀	25†	175.5	187	17	Weight	101.8	106.9	106.7	105.6	102.1	106.2	111.8
					Feed	61.8	100.0	97.1	88.2	85.3	85.3	117.7

All animals received 1 per cent NaCl solution to drink for the first 3 days following adrenalectomy. The comparatively low food intake during this period is an effect of the operative procedure.

Animals were 10 weeks old at operation.

* Received only water to drink after 3rd day after operation.

† Received 1 per cent NaCl solution to drink exclusively after operation.

‡ Weight in per cent of weight at operation.

§ Average food consumed daily during period indicated, in per cent of average food consumed daily 6 days before operation.

** Died at 20.3 days.

†† Died at 15 days.

which was water. Table 2 gives in detail the weight and food intake changes accompanying one per cent sodium chloride solution withdrawal and subsequent restoration in two cases.

In three instances animals were given water to supplant 1 per cent sodium chloride solution, and during the second three day period following the change each received 250 mgm. of sodium chloride daily by subcutaneous injection of a sodium chloride solution containing 100 mgm. of sodium

chloride per cubic centimeter of solution. The amount of sodium chloride received by these animals is less than one-half the amount taken in by adrenalectomized rats having free access to food and one per cent sodium chloride solution. Hence, it was not expected that the food intake would be elevated to within the bounds of normal intake range. However, all three animals reacted with an appreciable rise in food intake. Table 3 gives the data from two of these animals, the same two from which the data of table 2 had previously been gathered.

Normal rats treated in these ways with sodium chloride manifest no appetite changes.

TABLE 2

Weight and food intake changes accompanying replacement of 1 per cent NaCl solution by water and subsequent restoration of 1 per cent NaCl solution

		WEIGHT 3RD DAY BEFORE REPLACEMENT OF 1 PER CENT NaCl SOL. BY WATER	WEIGHT AT TIME OF REPLACEMENT	AVERAGE FOOD CONSUMED DAILY, 3 DAYS BEFORE REPLACEMENT		DAYS AFTER REPLACEMENT OF 1 PER CENT NaCl SOLUTION BY WATER								
						3	6	9		10	12	15	18	21
		grams	*grams*	*grams*										
♂	43	285	287.5	20	Weight*	94.3	87.3	80.4‡	1 per cent NaCl sol.	82.1	87.3	92.2	96.2	102.8
					Feed†	42.5	17.5	12.5		85.0	87.5‖	110.0	112.5	122.5
♀	23¶	205	206.5	14.5	Weight	87.9	87.2	83.1§		94.2	96.2	100.2		
					Feed	34.5	44.8	51.7		106.9	110.3‖	138.0		

These rats were 16 weeks old at beginning of experiment, and had been adrenalectomized 6 weeks previously.

* Weight in per cent of weight at time of replacement of 1 per cent NaCl solution by water.

† Average food consumed daily during period indicated, in per cent of average food consumed daily 3 days before replacement of 1 per cent NaCl solution by water.

‡ 1 per cent NaCl solution restored. In following 1 hour animal drank 20 cc., ate 7.5 per cent.

§ 1 per cent NaCl solution restored. In following 1 hour animal drank 28 cc., ate 17.2 per cent.

¶ Eighteen weeks after adrenalectomy this animal succumbed to replacement of 1 per cent NaCl solution by water in 7 days.

‖ 9th-12th day.

3. *A comparison between the desire for food of untreated and of sodium chloride treated adrenalectomized rats.* This experiment was performed to determine if the difference in food intake of these two types of experimental animal is a reflection of a difference in desire for food.

Four groups of rats were used. Group a was composed of eight fasted blank adrenalectomized rats drinking water; group b, of eight fasted adrenalectomized rats drinking water; group c, of seven fasted adrenalectomized rats drinking one per cent sodium chloride solution; and group d, of six fasted adrenalectomized rats drinking water and receiving 200 mgm. of sodium chloride per day by two subcutaneous injections of a solution containing 100 mgm. of sodium chloride per cubic centimeter of solution.

In table 4 sodium chloride intake, weight change and survival are shown for one male and one female from each group. The average survival of males and females of each group is also given. Within the male and female subdivisions of each group the weight changes are quite consistent, but the survival times are variable.

The appetite of an animal was tested by placing 2 grams of food before it. The time in which the animal consumed the food was taken as a measure of appetite although when an animal was obviously quite weak its interest in the food and its persistence in attempting to eat were taken into consideration. Animals of group a were so tested every third day following removal of food; animals of the other groups on the sixth day after removal of food and every third day thereafter. Animals of group a exhibited good

TABLE 3

Weight and food intake changes accompanying replacement of 1 per cent NaCl solution by water, followed in 3 days by NaCl administered subcutaneously

		DAYS AFTER REPLACEMENT OF 1 PER CENT NaCl SOLUTION BY WATER	
		3	6*
♂ 43	Weight†	93.3	95.3
	Feed‡	33.3	66.7
♀ 23	Weight	89.2	93.7
	Feed	40.7	62.9

These rats had been adrenalectomized 12.5 weeks previous to beginning of experiment.

* 250 mgm. of NaCl subcutaneously daily from 3rd to 6th day.

† Weight in per cent of weight at time of replacement of 1 per cent NaCl solution by water.

‡ Average food consumed daily during period indicated, in per cent of average food consumed daily 3 days before replacement of 1 per cent NaCl solution by water.

appetites. Animals of groups c and d displayed good appetites but were generally weaker than animals of group a and did not as a rule eat as rapidly. Animals of group b exhibited poor appetites. They lacked initial and protracted interest in the food, and if they did finish required a much longer time to do so than did animals of groups c and d. This appetite difference between untreated and sodium chloride treated adrenalectomized animals occurred in the face of equal susceptibility to fasting.

4. *The effect of food deprivation upon blank adrenalectomized rats and upon sodium chloride treated adrenalectomized rats.* It was desired to know to what extent the self-imposed fast of untreated adrenalectomized animals would affect the general condition of intact animals and of sodium chloride treated adrenalectomized animals; and if sodium chloride, in the absence of normal food intake, would alleviate adrenal insufficiency.

One animal of each of two pairs of male and two pairs of female littermates was adrenalectomized and after the recovery period had free access

TABLE 4

Weight change and survival of fasted blank adrenalectomized rats, and of fasted untreated and NaCl treated adrenalectomized rats

GROUP			AGE AT OPERATION	WEIGHT AT OPERATION	WEIGHT IN PER CENT OF WEIGHT AT OPERATION, 3 DAYS AFTER OPERATION, FOOD REMOVED		DAYS AFTER REMOVAL OF FOOD				SURVIVAL OF GROUPS IN DAYS AFTER START OF FAST, AVERAGE AND RANGE
							3	6	9	12	
			weeks	*grams*							
a	♂	21	24.5	420	97.0	NaCl*	0	0	0	0	>15.0
						Weight†	87.4	80.1	73.3	66.5	(all)
b	♂	14	19.5	356	97.7	NaCl	0	0	0		11.5
						Weight	88.1	80.9	76.1‡		(9.0–17.5)
c	♂	8	19.0	373	96.6	NaCl	217	140	167	30	11.0
						Weight	88.5	80.3	72.5	70.2§	(9.5–13.0)
d	♂	16	20.5	364	96.7	NaCl	233	200	200	200	11.0
						Weight	89.4	81.5	75.3	74.6¶	(9.5–12.5)
a	♀	14	23.0	206	96.1	NaCl	0	0	0		8.5
						Weight	84.0	74.5	61.1‡		(7.5–10.0)
b	♀	6	25.0	223	97.3	NaCl	0	0	0		8.5
						Weight	85.7	79.7	74.2‖		(5.5–9.5)
c	♀	1	24.0	218	99.1	NaCl	140	107	90	110	10.5
						Weight	87.7	82.4	79.8	77.5§	(6.5–14.5)
d	♀	10	25.5	240	98.1	NaCl	233	200	177		8.5
						Weight	90.0	82.8	73.7‡		(8 and 9)

* Average daily intake of NaCl in milligrams during period indicated.

† Weight in per cent of weight at time of food removal.

Group a, blank adrenalectomized on water; group b, adrenalectomized on water; group c, adrenalectomized on 1 per cent NaCl sol; group d, adrenalectomized on subcutaneous injection of NaCl.

‡ Lived 9 days.

§ Lived 10 days.

¶ Lived 9.5 days.

‖ Lived 8.5 days.

to food and water. The other animal of each pair was blank adrenalectomized one day later and after the recovery period received water to drink

and the amount of food daily consumed by its adrenalectomized littermate. At operation the animals were 10 weeks old, and the members of each pair had very nearly the same weight and food intake. The blank adrenalectomized animals lost weight very slightly faster than the adrenalectomized animals, but out-lived them.

Five adult male rats (group 1) were adrenalectomized and were allowed 0.1 per cent sodium chloride solution or water to drink and food ad lib. after the recovery period. Four adult male rats (group 2) were adrenalectomized one day later and after the recovery period received one per cent sodium chloride solution to drink and each was allotted daily the average amount of food eaten by the surviving animals of group 1. Food intake of animals of group 1 fell at the beginning of the experiment and dropped sharply to very low levels from two to five days prior to death. Three deaths in this group occurred at intervals to the end of the 19th day, at which time the remaining two animals did not yet display the extremely low food intake which always precedes death. These circumstances, coupled with the fact that the animals of group 2 always ate immediately the food allotted them, was interpreted to mean that animals of group 2 would not be subjected to the very low food intake leading to death till the two remaining rats of group 1 approached death. Hence, beginning the 20th day the rats of group 2 were allotted a limited volume of 2 per cent sodium chloride solution and small quantities of food such as taken by untreated adrenalectomized rats when their food intake has dropped to the level of extreme privation two to five days before death. During this period the animals ingested 200 mgm. of sodium chloride daily. They died in from two to five days after the start of the period. The sodium chloride which they had received from the time of operation to death had sustained their appetite, but had left them just as susceptible to inanition as are untreated animals.

One animal of each of three groups of males and three groups of females was adrenalectomized and after the recovery period was allowed food and water ad lib. A second animal of each group was adrenalectomized and after the operation received 1 per cent sodium chloride solution and the same amount of food daily in proportion to its previous intake as that consumed by the first animal. The third animal of each group was blank adrenalectomized and after the operation received water and the same amount of food daily in proportion to its previous intake as that consumed by the first animal. All the animals were 10 weeks old at the time of operation. Table 5 contains a summary of the pertinent data of this experiment. It will be noticed that two out of the six normal animals and three out of the five salt treated adrenalectomized animals succumbed to the low food intake sooner than did their untreated adrenalectomized partners.

With a few exceptions occurring soon after operation, all the adrenalec-

TABLE 5

Survival and final weight change of adrenalectomized rats drinking water, adrenalectomized rats drinking 1 per cent NaCl solution, and blank adrenalectomized rats drinking water; with members of each group on comparable food intakes

	ADRENALECTOMIZED. WATER	ADRENALECTOMIZED. 1 PER CENT NaCl	BLANK ADRENALECTOMIZED. WATER
Females. Group 1			
Weight after 12 days in per cent of weight at operation	81.4	83.0	77.7
Survival in days	15.0	13.0	>15.0
Weight at above time in per cent of weight at operation	75.6	80.7	67.3
Females. Group 2			
Weight after 6 days in per cent of weight at operation	82.9	86.1	67.9
Survival in days	10.7	>10.7	7.5
Weight at above time in per cent of weight at operation	70.5	69.3	60.3
Females. Group 3			
Weight after 12 days in per cent of weight at operation	89.1	87.3	84.5
Survival in days	14.0	13.0	>14.0
Weight at above time in per cent of weight at operation	84.0	84.6	82.3
Males. Group 1			
Weight after 12 days in per cent of weight at operation	82.1	80.6	63.8
Survival in days	20.3	18.0	14.0
Weight at above time in per cent of weight at operation	65.4	70.7	56.4
Males. Group 2			
Survival in days	17.3	>17.3	>17.3
Weight at above time in per cent of weight at operation	73.4	83.3	74.7
Males. Group 3			
Survival in days	15.3		>15.3
Weight at above time in per cent of weight at operation	78.7		74.0

tomized rats drinking one per cent sodium chloride solution and receiving daily food allotments finished very quickly the food which untreated adrenalectomized rats had required 24 hours to eat.

Discussion. Data reported here show that the progressive decline and the daily fluctuations of body weight which occur in sodium chloride treated and in untreated adrenalectomized rats on subnormal intake levels are normal responses to the amount of food consumed.

Untreated adrenalectomized animals have a low fluid intake, but this is a normal concomitant of their low food intake. Comparably starved normal animals reduce their fluid intake to the same extent. Though untreated adrenalectomized animals have a low fluid intake due to their low food intake, adrenalectomized animals receiving the same amount of food but with one per cent sodium chloride solution to drink have a somewhat higher fluid intake. In experiment 3, the fluid intake of animals of group d was much higher than that of animals of group b; and in the case of the males slightly lower, in the case of the females slightly higher than in animals of group c.

It is clear that untreated adrenalectomized rats are subjected to a self-imposed starvation, occasionally of a degree severe enough to be quickly fatal even to normal rats. As regards survival, sodium chloride treatment does not help fasted adrenalectomized rats, or even adrenalectomized rats receiving food in the amount eaten by untreated adrenalectomized animals. That sodium chloride favorably affects the appetite of the adrenalectomized rat is adequately shown by the foregoing set of experiments. Certainly, a large part of the ability of sodium chloride to maintain life in the adrenalectomized rat resides in its action in sustaining the appetite of the animal so that its food intake is normal.

Treatment of adrenalectomized rats drinking water with ascorbic acid, thiamin chloride hydrochloride, or adrenaline does not prevent the loss of appetite.

Selye (1936) has shown that untreated adrenalectomized rats are very susceptible to drugs (morphine, atropine, adrenaline). Adrenalectomized rats allowed 0.9 per cent sodium chloride solution to drink and food ad lib. showed almost complete restoration of resistance. Adrenalectomized rats allowed 0.9 per cent sodium chloride solution to drink and no food were just as susceptible to drugs as were untreated animals. These experiments were performed two days after adrenalectomy. Selye also mentioned that Dr. J. S. L. Browne, in a personal communication, wrote that in cases of Addison's disease salt treatment was efficient only if the patient took sufficient nourishment.

The effects of adrenalectomy upon fluid and electrolyte distribution and upon carbohydrate and related protein metabolism are generally looked upon as being of prime importance among the symptoms of adrenal in-

sufficiency. The work of Swingle et al. (1937a, 1937b), Silvette (1934), Silvette and Britton (1935, 1936), and Hartman et al. (1940a, 1940b) strongly indicates that serum sodium and chloride ion levels and loss of these two ions through the urine are of little real significance in adrenal insufficiency. It follows from a study of the work referred to that the loss of appetite following adrenalectomy cannot be ascribed to the lowered serum sodium and chloride ion concentrations, and that the effect of sodium chloride upon the appetite would not seem to be mediated simply through the elevation of these two ions in the extracellular fluid. Long et al. (1940) showed that adrenalectomized mice and rats treated with sodium salts and allowed to eat have normal liver glycogen content, though the blood glucose level of the rats is slightly below normal. Liver glycogen and blood glucose drop considerably in normal animals fasted 24 and 48 hours, but the fall is still greater in adrenalectomized animals even though they have been treated with sodium salts. Adrenalectomized fasted rats, though salt treated, occasionally develop hypoglycemic convulsions after a 48 hour fast. Following glucose administration, the rats untreated with salt get transitory relief but death soon follows. The salt treated rats are likewise relieved by glucose administration, but they then eat and remain well (Long). Thus, the anorexia after adrenalectomy does not seem to result from the lowered liver glycogen and blood glucose, and sodium chloride treatment does not maintain appetite through a restorative effect on the disturbed carbohydrate metabolism.

The question of the causes of the loss of appetite is no more important than the question of the effects of the loss. In cases of chronic adrenal insufficiency the possibility of complication by multiple nutritional deficiencies is readily apparent in view of the quantitative study reported here. Much less obvious, but yet of paramount significance, is the possibility of nutritional deficiency involvements occurring in animals very rapidly after withdrawal of cortical secretion and food. An adrenocortical insufficient animal, untreated, is victim of two primary deficiencies, lack of cortical secretion and lack of nutriment. It is not at all logical to deduce the symptoms which would result from lack of cortical secretion alone by simply subtracting from the total the symptoms displayed by the intact nutriment deficient animal. Except for an extremely short time after withdrawal of cortical secretion, the symptoms of the animal should be considered as a reflection of a single physiological state resulting from a blending of the two deficiencies. Of course, it is possible to determine what symptoms accrue from superimposing cortical secretion deficiency upon nutriment deficiency, but this does not necessarily provide a clue to the normal function of the secretion. To determine the normal function unequivocally requires a secretion deficient animal with a normal food intake. On the other hand, it is perfectly plausible to identify certain aspects of cortical-nutriment de-

ficiency as being predominantly referable to the nutriment deficiency, since a nutriment deficiency in the presence of a cortical sufficiency is a producible condition. The demonstration of qualitative differences in the physiological action of certain compounds obtained from the adrenal cortex (Wells and Kendall, 1940, and Ingle, 1940) in no way vitiates these considerations.

SUMMARY

1. Untreated adrenalectomized rats eat very little food. Treatment with sodium chloride maintains food intake within the normal range.

2. Treatment of adrenal insufficient rats with sodium chloride results in a suddenly renewed interest in food.

3. Fasted untreated adrenalectomized rats show little desire for food, in contrast to the behavior of fasted sodium chloride treated adrenalectomized rats.

4. Salt treated adrenalectomized rats and even blank adrenalectomized rats may succumb sooner than untreated adrenalectomized rats when subjected to the fast which the latter undergo voluntarily.

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STUDIES ON THE METABOLISM OF PANTOTHENIC ACID IN MAN AND RABBITS[1]

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The physiological importance of pantothenic acid for microörganisms and Vertebrata, together with its universal distribution in animal tissues has been adequately reviewed by Williams (1941). Evidence has been presented (Spies et al., 1940; Stanbery et al., 1940) on the basis of blood and urine studies indicating that restricted human diets leading to a pellagrous condition may be deficient in pantothenic acid.

The pantothenic acid content of whole blood, plasma and cells of various mammalian species has been studied by Pearson (1941). Pelczar and Porter (1941) have studied the pantothenic acid content of human blood and urine. Their figures for human blood which range from 3.0 to 9.9 μgm. per cent are much lower than the average value of 22.5 μgm. per cent reported by Stanbery et al. (1940) and 19.4 μgm. per cent reported by Pearson (1941). Presumably this difference is due to the microörganisms employed in the assay. Pelczar and Porter used *Proteus morganii* while Stanbery et al. and Pearson used *Lacto bacillus casei* ε based on the method of Pennington et al. (1940). Since the latter organism has been used extensively in the assay for riboflavin in biological materials including blood and urine and its adequacy as a test organism for pantothenic acid well established, comparisons cannot be satisfactorily made between results obtained with this and other organisms when the results differ so markedly. The results of the studies on the pantothenic acid content of blood using *Proteus morganii* as the test organism does, however, suggest, as pointed out by the authors, that pantothenic acid may exist in the blood in free and conjugated forms and that the latter may be incompletely available to this organism.

It has previously been reported (Pearson, 1941) that pantothenic acid occurs in approximately equal concentration per 100 ml. of plasma and cells. There is a species variation. In the dog and pig the plasma contains more pantothenic acid per 100 ml. than the cells, while in the horse, human,

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rabbit and sheep the concentration is greater in the cells. The present investigation deals with the effect of massive doses of Ca-pantothenate on the amount in the blood, distribution between plasma and cells, and the rate of excretion in the urine.

EXPERIMENTAL. *Pantothenic acid in whole blood, plasma and cells.* Normal rabbits weighing between 1.6 and 2.0 kgm. were used for these studies. The standard stock diet which they had received since weaning was continued during the experimental period. Massive doses of Ca-pantothenate were administered either orally or by parenteral injection just underneath the skin. Blood for assay purposes was obtained directly from the heart. Pantothenic acid was determined by the method described by Stanbery et al. (1940) and the plasma and cells were treated as described by Pearson (1940). Approximately two weeks prior to the administration of the test dose a blood sample was taken for the determination of normal pantothenic acid values. The intervening period of about two weeks was allowed for the animals to recover from the loss of blood which amounted to approximately 15 ml.

Dextrorotatory calcium pantothenate, hereafter referred to as *Ca-pantothenate,* was used in all of the experiments reported in this paper. The term *pantothenic acid* in blood and urine as used in this paper refers to the values obtained with Ca-pantothenate used as the standard.

The data on the effect of the administration of large doses of Ca-pantothenate on the amount in whole blood, plasma and cells, and the ratio of the amount in the plasma to that in the cells are summarized in table 1. Ca-pantothenate administered either orally or parenterally is rapidly absorbed into the blood stream. Sixty minutes after the injection of 500 mgm. of Ca-pantothenate into rabbit 1, the level in the blood increased to 6400 μgm. per cent, which is equivalent to a 114-fold increase above the normal value. The amount in the blood of rabbit 2 showed an even greater increase when the blood was drawn 75 minutes after injection of the test dose. When only 400 mgm. of Ca-pantothenate was injected into rabbit 6 the level in the blood 90 minutes later was 4320 μgm. per cent. It is possible that this might have been after the amount in the blood had begun to decline and, as is pointed out later in the paper, pantothenic acid is readily eliminated from the body. When Ca-pantothenate was administered orally the increment in the level in the blood was much less than when injected. Ninety minutes after the ingestion of 500 mgm. by rabbit 3 the blood contained 487 μgm. per cent of pantothenic acid which is approximately a 10-fold increase over the normal level.

There is a relatively greater increase in the amount of pantothenic acid in the plasma than in the cells following the administration of a large dose. This fact is evinced from the last column of table 1 which gives the ratio of microgram per cent plasma pantothenic acid to microgram per cent cell

pantothenic acid. In the normal animal this ratio fluctuates around one, while in the injected animals the amount of pantothenic acid in the plasma is 4.9 to 12 times the level in the cells. Rabbit 2 which shows the greatest increase in blood pantothenic acid likewise had the widest plasma/cell pantothenic acid ratio. This relationship is not unexpected as it is natural that constituents which are rapidly eliminated from the body by the kidney should occur principally in the plasma. In a general way the higher pantothenic acid values in the blood following the administration of the test dose tend to be accompanied by a wider plasma/cell pantothenic acid ratio.

TABLE 1

The effect of the administration of massive doses of Ca-pantothenate on the amount in whole blood, plasma and cells

RABBIT NO.	Ca-PANTOTHENATE ADMINISTERED	PANTOTHENIC ACID PER 100 ML.			PLASMA P. ACID / CELL P. ACID =
		Whole blood	Plasma	Cells	
		γmg.	*γgm.*	*γgm.*	
1	None	56	47	58	0.81
	60 minutes after injection of 500 mgm.	6400	9300	1800	5.17
2	None	75	74	75	0.99
	75 minutes after injection of 500 mgm.	10000	14400	1200	12.00
3	None	48	50	39	1.28
	90 minutes after ingestion of 500 mgm.	487	680	131	5.19
5	None	74	88	68	1.29
	120 minutes after ingestion of 400 mgm.	377	470	113	4.16
6	None	92	82	100	0.82
	90 minutes after injection of 400 mgm.	4320	5640	1150	4.90

Urinary excretion of pantothenic acid. Rabbits were first used in studying the rate of excretion and recovery of Ca-pantothenate administered in large doses. It was soon apparent that rabbits were not very satisfactory for this purpose as they micturated very infrequently, especially during the day; most of the urine was excreted during the night, as is common with nocturnal animals. The data collected on two rabbits and recorded in table 2, afford some indication of the amount of pantothenic acid excreted by this species during a 24-hour period.

Prior to the administration of the test dose the urine was collected under toluene for 48 consecutive hours. The urine was taken from the metabolism cage twice daily and stored in the refrigerator preparatory to analysis. Rabbit 5 which received 400 mgm. of Ca-pantothenate per os ex-

creted in the urine during the succeeding 24 hours 211.4 mgm. of pantothenic acid. When correction is made for the amount excreted over a corresponding period on the basal diet this amounts to a net recovery of 52 per cent of the amount ingested. When the same amount of Ca-pantothenate was injected there was a net recovery of 295.3 mgm. in the urine or approximately 74 per cent.

The higher amount of pantothenic acid recovered in the urine after parenteral administration is in accord with the higher levels in the blood when the test dose is injected as compared with the figures for blood and urine when the test dose is ingested. The percentage recovery in the urine of ingested Ca-pantothenate is much higher in the rabbit than in man. However, on the basis of body weight the amount ingested was approximately 12 times greater in the rabbit than in the human subjects.

Since it was desirable to collect urine samples at relatively frequent and regular intervals and to obtain a blood sample at the end of each period, it

TABLE 2

Urinary excretion of pantothenic acid by rabbits during 24-hour period

RABBIT NO.	Ca-PANTOTHENATE ADMINISTERED	PANTOTHENIC ACID EXCRETED	
		Total	Net recovery
		mgm.	*mgm.*
5	None	1.3	
	400 mgm. orally	211.4	210.1
6	None	1.1	
	400 mgm. injected	296.3	295.2

was evident from experience with the rabbits that human subjects would be much more satisfactory. Healthy men presumably on an adequate diet served as the experimental subjects. The general plan was to collect the urine for 48 hours prior to the administration of the test dose. The test dose of one gram of Ca-pantothenate in aqueous solution was taken at 6 a.m. just after the bladder had been emptied. The urine was then collected for the periods indicated in table 3 and a blood sample taken at the end of each period.

While there is a marked variation in the amount of pantothenic acid excreted during the respective periods and the total 26 hours, an inspection of table 3 reveals definite consistencies in the response of the three individuals to the test dose. The amount of pantothenic acid excreted during the 48-hour period while on the normal diet was 6.32, 6.60 and 6.23 mgm. for subjects A, B and C respectively. These figures are surprisingly close considering the fact that the meals of each of the individuals were prepared at their respective places of abode. These values for the daily excretion of panto-

thenic acid are of the same order as those reported by Pelczar and Porter (1941). The fact that the urinary values for pantothenic acid reported by these authors are in accord with those reported in this paper substantiates the suggestion made at the beginning of this paper, namely, that the wide difference in the blood pantothenic acid values reported by Pelczar and Porter, and other workers (Stanbery et al., 1940; Pearson, 1941) may be due to the fact that a portion of the pantothenic acid of the blood exists in a

TABLE 3

Rate of excretion of pantothenic acid and amount in human blood

SUBJECT	Ca-PANTOTHENATE INGESTED	HOURS AFTER INGESTION	COLLECTION PERIOD	PANTOTHENIC ACID EXCRETED		BLOOD PANTOTHENIC ACID
				Per period	Per hour	
	mgm.		*hours*	*mgm.*	*mgm.*	*μgm. per cent*
A	None		48	6.32	0.13	28.0
	1000	2	2	16.48	8.24	46.0
		5	3	6.84	2.28	41.0
		8	3	2.64	0.88	36.0
		11	3	1.03	0.34	36.0
		26	15	4.63	0.31	34.0
Total				31.62		
B	None		48	6.60	0.14	18.0
	1000	2	2	78.30	39.15	112.0
		5	3	22.76	7.59	72.0
		8	3	10.00	3.33	54.0
		11	3	3.74	1.25	42.0
		26	15	5.76	0.38	30.0
Total				120.56		
C	None		48	6.23	0.13	20.0
	1000	2	2	31.50	15.75	78.0
		5	3	14.62	4.87	46.0
		8	3	12.87	4.29	37.0
		12	4	5.26	1.31	34.0
		26	14	6.82	0.49	29.0
Total				71.08		

form which is incompletely available to *Proteus morganii*, the organism used by Pelczar and Porter.

That the Ca-pantothenate is rapidly absorbed and then eliminated by the kidney is apparent from the fact that the maximum rate of excretion of pantothenic acid in the urine occurs during the first two hours following the ingestion of the test dose. Calculated on the amount of pantothenic acid excreted per hour there is a rapid decline after the first collection period of

two hours. During this period the hourly rate of excretion was 8.24, 39.15 and 15.75 mgm. for subjects A, B and C respectively. While there is a marked difference in these figures, an examination of the data shows that the proportionate rate of decline is of the same order for each individual. Thus the ratio of the hourly rate of pantothenic acid excretion between the first and second periods is 3.6, 5.1 and 3.2 for subjects A, B and C respectively. The ratio between the hourly rate of pantothenic acid excretion during the last period of 15 hours for subjects A and B and 14 hours for subject C and the rate on the basal diet was 2.4, 2.7 and 3.8 respectively. On the basis of the rate of excretion it is apparent that by far the greater part of the pantothenic acid eliminated occurs by way of the renal pathway during the first 26 hours. As a matter of fact more than 50 per cent of the total recovered was excreted during the first two hours after ingestion of the test dose. From these data there is no reason to believe that appreciable amounts of pantothenic acid would have been recovered had the urine been collected for an additional 24 hours.

Relationship of blood pantothenic acid to rate of excretion in the urine. There was considerable variation in the maximum levels of pantothenic acid in the blood following the test dose. However, the values for each individual as correlated with the amount in the blood is fairly consistent. The maximum levels were observed at the end of the first 2-hour period after the administration of the test dose. It is, of course, recognized that the blood may not have been drawn at just the moment the pantothenic acid content of the blood was at its highest level. Nevertheless, the maximum level observed in the blood and the maximum rate of excretion both occurred during the first 2-hour period. The highest level of pantothenic acid observed was 112.0 μgm. per cent for subject B, and this individual likewise had the highest rate of excretion during this period. In subject A the maximum level of pantothenic acid observed in the blood was only 46.0 μgm. per cent while the maximum hourly rate of excretion in the urine was 8.24 mgm. In each individual the amount of pantothenic acid in the blood continued to decrease with each successive period as did the rate of excretion in the urine. However, at the end of 26 hours none of them had returned to their original values.

It is of passing interest to compare the pantothenic acid content of the blood of these individuals on normal diets with the amounts found four months previously (Pearson, 1941). The levels observed at that time were 23.0, 16.0 and 21.0 μgm. per cent respectively for subjects A, B and C as compared with 28.0, 18.0 and 20.0 μgm per cent as reported in table 3. While the present values are not identical with the earlier values they do occupy the same relative positions.

Recovery of ingested Ca-pantothenate. There are at least two possible ex-

planations of the wide difference in the response of the three individuals after ingestion of the test dose in respect to the amount recovered in the urine, maximum rate of excretion and maximum amount of pantothenic acid in the blood. The response may be due to a difference in the amount of the test dose that was absorbed. The data also suggest that the capacity for storage of absorbed pantothenic acid may have differed in the experimental subjects. Perhaps the diet of individual A had not furnished as much pantothenic acid as had the diet of B and C. Thus the tissues of subject A were less saturated with pantothenic acid resulting in a higher per cent being retained while B's tissues were more saturated and thus had less capacity for storage of the acid. This theory, however, is not supported by a difference in the daily excretion of pantothenic acid by the individuals while on normal diets.

The percentage recovery of ingested Ca-pantothenate was relatively low in the experiments with humans. After correction is made for the excretion in the urine on the basal diet the net recovery of ingested Ca-pantothenate was 2.8, 11.7 and 6.8 per cent for subjects A, B and C respectively. Interest naturally arises in what happened to the pantothenic acid not recovered in the urine. For the present this question remains unanswered. Of course a large proportion of the pantothenic acid may have been stored in the tissues, especially in the liver. If this were the case it was certainly removed very rapidly from the blood. Another possibility is that the pantothenic acid was converted to other compounds, such for example as the conversion of the alanine moiety to glucose. Analysis of the tissues for pantothenic acid would be very helpful in advancing our knowledge on the metabolism of the pantothenic acid which was not recovered in the urine.

There was no evidence of toxicity following the administration of large doses of Ca-pantothenate. Nor were there unfavorable effects such as flushing of the skin as results from the administration of nicotinic acid (Sebrell and Butler, 1938; Popkin, 1939).

SUMMARY

1. Data are reported on the effect of the administration of large doses of Ca-pantothenate on the pantothenic acid content of whole blood, plasma and cells, and the rate of excretion in the urine.

2. Following the administration of a large dose of Ca-pantothenate there is a rapid increase in the amount in the blood. The extent of the increase appears to depend upon the size of the dose per unit of body weight and the mode of administration. The increment of pantothenic acid in the blood is more marked when the test dose is administered parenterally than when it is given orally. The ratio between the pantothenic acid content of the plasma and cells following the administration of the test dose was from 4.2 to 12.0 as compared with a ratio of approximately one for normal animals.

3. There is a definite relationship between the amount of pantothenic acid recovered in the urine and the maximum level observed in the blood.

4. Over 50 per cent of the total pantothenic acid recovered in the urine of humans following the administration of the test dose occurred during the first two hours. Following the initial 2-hour period there was a rapid decrement in the rates of excretion of pantothenic acid which was concomitant with a decrease in the amounts in the blood.

5. When 400 mgm. of Ca-pantothenate was administered orally to a rabbit 52 per cent was recovered in the urine within 24 hours. When the same amount was administered parenterally 74 per cent was recovered in the urine during the corresponding period.

6. The percentage recovery of pantothenic acid in the urine of rabbits was much greater than for man; however, the amount ingested by the latter was only about one-twelfth the dose administered to rabbits on the basis of body weight. Of 1 gram of Ca-pantothenate ingested by humans 7.8, 11.7 and 6.8 per cent for the respective subjects was recovered in the urine during the succeeding 26 hours.

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SOME EFFECTS OF SULFANILAMIDE ON MAN AT REST AND DURING EXERCISE

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The effects of sulfanilamide on the acid-base balance of man have been repeatedly studied. It is well established that the drug produces a fall in the CO_2-combining capacity of the blood with a compensatory decrease in the alveolar pCO_2 and an increase in lung ventilation. Beckman et al. (1) attribute these changes to deficient reabsorption of bicarbonate by the kidney tubules. Hartmann et al. (2), however, regard the hyperpnea as a central effect occurring earlier than would be possible in response to excess excretion of bicarbonate in the urine, though in the dog McChesney et al. (3) recently claim to have shown that the kidney changes come first in order of time. In search for an explanation of the lowered bicarbonate of the blood, Mann and Keilin (4) have studied the effect of sulfanilamide on dilute solutions of the enzyme carbonic anhydrase and have discovered a specific poisoning effect by the drug at concentrations of less than 1 mgm. per cent. In man at rest, however, no appreciable changes have been observed in the rate of CO_2 output in the expired air and but little in the pH of the plasma even when the blood contains several milligrams per cent of sulfanilamide. The body can presumably therefore compensate against depression of the rate of CO_2 output *in vivo* by sulfanilamide. One object of the present work was to elucidate the nature of this mechanism and to see, by metabolic and blood studies under two grades of muscular exercise as well as at rest, how far it remains effective when the processes for CO_2 removal are put to increasing strain. So far as we know, the effects of sulfanilamide in moderate and severe exercise have not been investigated before, though they are of obvious practical importance in deciding how much work can be done by industrial and military personnel while under treatment by the drug. Not only is sulfanilamide widely used for ambulant patients, particularly those suffering from venereal disease, but its use has also been proposed as a prophylactic measure. Our second and equally important object was therefore to find out how much physical and mental handicap is induced by moderate doses of sulfanilamide. We have accordingly made psychological and cardiovascu-

lar measurements to supplement the customary metabolic and blood studies. These four sets of data will first be presented, since they yield a fairly complete answer to the practical question just mentioned and do also throw some light on how the body compensates against the expected depression in the rate of CO_2 output by sulfanilamide. This more difficult problem, however, requires in addition new data on the effect of sulfanilamide on 1, the activity of carbonic anhydrase in the red cell rather than in the dilute solutions studied by Keilin and Mann; 2, the CO_2 pressures in the alveolar air and the arterial and mixed venous bloods. This part of the research is as yet incomplete, but the results and conclusions so far obtained will be given in the latter part of this paper.

EXPERIMENTAL PROCEDURE. Six normal subjects were given two to three grams of sulfanilamide daily. Observations were made before and during the drug administration on the basal gas exchange, pulse, blood pressure, electrocardiogram, and CO_2 and O_2 (5) in the arterial blood. The arterial blood was analyzed for CO_2 and O_2, and a part of the blood sample was equilibrated at 37°C. with a gas mixture of $pCO_2 = 40$ and $O_2 = 200$ mm. Hg. From these analyses the pCO_2 and pHs of the arterial blood were calculated (Dill, Graybiel, Hurtado and Tacquini, 6). Alveolar gas samples and samples of rebreathed CO_2 mixture also were obtained to estimate the arterio-venous pCO_2 differences. Similar observations were made in exercise on the bicycle ergometer, the oxygen consumption during the exercise being 5 to 6 times the basal level. Finally the subjects ran to exhaustion on an inclined, motor-driven treadmill while determinations were made of maximum pulse, oxygen consumption and CO_2 output. EKG and blood pressure were recorded after the run.

Three types of psychological tests were made daily on three of these subjects before, during and after sulfanilamide. At the same time two control subjects, not receiving the drug, were similarly tested. The tests used were the Johnson Code Test, the Woodworth-Wells Form-Naming Test, and the Pursuit Meter.

Sulfanilamide concentration in the blood (7) was determined daily, likewise the amount of sulfhemoglobin and methemoglobin.

Carbonic anhydrase activity of the intact red cells was estimated as described later.

Three of the subjects (D. B. D., F. C. and J. T.) receiving 3 grams of sulfanilamide daily and the one (N. R.) receiving 2 grams daily were able to continue the complete tests for at least two successive days while taking the drug. However, F. C. vomited once on the second day and N. R. was dizzy and nauseated on the third day. R. D. took 3 grams daily for $3\frac{1}{3}$ days but did not go through the exhausting run, since he developed a head cold at the end of the second day. B. C. vomited on the first day, was nauseated and barely able to finish the bicycle exercise on the second day. The treadmill run and further studies on him were impossible.

The dose of sulfanilamide was chosen to correspond to the maximum customarily administered to ambulatory patients.

SECTION I. PHYSIOLOGICAL AND PSYCHOLOGICAL EFFECTS OF SULFANILAMIDE ON MAN. Table 1 presents a summary of some of the measurements in all six subjects for resting conditions and two degrees of exercise. The results in each subject are presented chronologically: first the control period and then the sulfanilamide period, finally in the cases of F. C. and J. T. an experiment in the first day of the recovery period. On each day of tests the blood sulfanilamide value is noted. Methemoglobin and sulfhemoglobin values were no more than a trace in any case and are omitted from the table.

Resting conditions. The measurements here merely confirm those previously noted by others. The regular changes are a drop in the blood CO_2 capacity (T_{40}), a compensatory decrease in arterial pCO_2 and an increase in ventilation. The change in ventilation is absent in subjects F. C. and B. C. but present in all others. There is some variability in basal oxygen utilization and CO_2 output but no definite trend except a rise in the case of R. D. who was suffering from an upper respiratory infection during the sulfanilamide period.

The pulse rate and blood pressure at rest showed no definite changes after sulfanilamide. However, electrocardiographic changes at rest were observed in five of the six subjects following sulfanilamide administration. In four cases there was well-marked lowering of the T waves predominantly in leads 1 and 2 or leads 2 and 3. These changes appeared within 24 hours of starting the drug and continued through the period of its administration. In one subject, B. C., who developed early severe toxic symptoms, the T waves became frankly inverted in lead 2 and remained so for the remaining 24 hours before the experiment was discontinued.

In the blood values the only additional points of note are the T_{40} values during recovery on F. C. and J. T. J. T. had taken 5 grams $NaHCO_3$ with each 1 gram of sulfanilamide. As soon as both were stopped, the sulfanilamide effect apparently persisted longer and so caused a further drop in CO_2 capacity. In the case of F. C., who received no $NaHCO_3$, the T_{40} value remained at its low level during the first 24 hours of recovery. Tests during the recovery period on these two subjects afford an opportunity to observe the effects of lowering of the bicarbonate without significant sulfanilamide in the blood.

Bicycle exercise. All except B. C. performed this exercise with ease after sulfanilamide, although it was somewhat harder for most of them. B. C. was barely able to finish the test.

In this moderate exercise the ventilation was invariably increased after sulfanilamide (average increase = 25 per cent). However, the rate of oxygen intake and CO_2 removal showed only minor variations. The

TABLE 1

Gaseous exchange and arterial blood values before and during administration of sulfanilamide

SUBJECT	BLOOD SULFANILAMIDE	RESTING CONDITION						BICYCLE EXERCISE							TREADMILL EXERCISE					
					Arterial blood							Arterial blood			2nd minute			3rd minute		
		Ventilation S.T.P.	CO_2/min.	O_2/min.	pCO_2	T_{co}	pH	Pulse per min.	Ventilation S.T.P.	CO_2/min.	O_2/min.	pCO_2	T_{co}	pH	CO_2	O_2	R.Q.	CO_2	O_2	R.Q.
	mgm. per cent	*l./min.*	*cc.*	*cc.*	*mm. Hg*	*vol. per cent*			*l./min.*	*cc.*	*cc.*	*mm. Hg*	*vol. per cent*		*l.*	*l.*		*l.*	*l.*	
D. B. D.	0	5.48	192	234	44.1	48.9	7.38	112	26.5	1175	1320	40.9	48.5	7.40	3.04	2.82	1.03	3.36	2.82	1.19
	0	5.73	192	227				104	26.7	1210	1340									
	2.4	6.60	229	248				128	35.0	1315	1455	38.4	42.4	7.34						
	4.2	6.64	207	246	37.3	44.7	7.39	124	40.4	1340	1510	34.2	40.5	7.37	2.17	2.57	0.84	2.63	2.79	0.94
	3.9	6.83	206	242				120	37.7	1215	1380				2.20	2.75	0.80	2.41	2.43	0.99
(NH_4Cl, 10 grams)	0					44.2									2.46	2.42	1.02	2.83	2.58	1.10
F. C.	0	6.08	180	235				108	28.4	1165	1415									
	0	6.99	194	235	45.5	48.6	7.37	124	29.2	1235	1435									
	0	7.42	204	251				116	27.3	1110	1290	41.0	48.3	7.41	3.12	3.27	0.96	3.68	3.36	1.10
	2.7	6.89	190	249	41.5	46.8	7.38	120	30.1	1050	1280	41.0	44.5	7.37						
	3.3	7.40	194	258	36.8	44.3	7.39	144	34.2	1105	1330	36.3	41.6	7.39	3.13	3.37	0.92	3.48	3.50	0.99
(Recovery)	0.8	7.87	202	252		44.9									3.41	3.55	0.96	3.65	3.39	1.08
N. R.	0	4.96	180	221	44.8	47.7	7.37	132	23.0	1270	1375	44.9	42.8	7.33	3.03	3.03	1.00			
	0	4.64	180	215				128	22.3	1145	1290				3.09	2.94	1.06	3.22	2.86	1.13
	2.3	5.96	219	257	45.0	45.1	7.34	140	27.2	1235	1365	43.4	41.2	7.32	2.82	2.80	1.01	3.15	2.82	1.12
	3.6	5.24	182	228		43.6		132	28.8	1210	1385	43.4	38.5	7.30	2.62	2.83	0.93	3.01	2.88	1.05
J. T.	0	7.50	190	250	41.8	48.2	7.39	116	29.9	1090	1200	40.3	43.2	7.36	2.37	2.74	0.87	2.99	2.83	1.06
	2.9	8.02	188	249	40.8	45.7	7.37	128	36.4	1170	1380	40.7	42.1	7.36						
	3.1	8.04	195	265	35.0	44.8	7.40	144	37.9	1175	1350	35.7	40.7	7.38	2.31	2.95	0.78	2.69	2.65	1.01
(Recovery)	0.8	8.15	212	269		41.0									2.40	2.74	0.88	3.20	2.90	1.10
R. D.	0	6.10	231	290	38.3	50.0	7.44	134	33.6	1415	1605									
	0	5.66	203	271				132	32.7	1375	1510	38.0	45.7	7.35						
	3.3	6.85	240	310	37.4	45.1	7.39	148	44.2	1510	1710									
	2.8	6.58	234	296	33.6	44.7	7.42	158	40.3	1475	1650	39.8	39.4	7.31						
	2.9	6.93	241	310																
B. C.	0	5.89	198	231	45.8	49.7	7.37													
	0	6.11	202	238	46.4	50.0	7.37	120	32.5	1305	1460	46.6	45.7	7.33						
	2.6	5.84	171	219	40.3	45.3	7.37	152	40.5	1400	1525	38.4	38.6	7.32						

arterial bloods showed a similar change to that at rest, namely, a drop in bicarbonate (T_{40}) and a compensatory decrease in pCO_2.

The toxic effect of the drug on the cardiovascular system is more clearly evident during this exercise than at rest. Invariably a faster pulse rate was observed in the sulfanilamide period than in the control period, the rise ranging from 10/min. in D. B. D. and N. R. to 32 in B. C. Changes in blood pressure were significant only in D. B. D. and B. C. In D. B. D. it rose from 130/80 to 162/90; in B. C. from 162/90 to 190/100.

Electrocardiograms taken immediately after the bicycle exercise were usually difficult to compare with those after similar exercise before administering sulfanilamide, due to the faster heart rate usually present in the former instance. While in such conditions an increase in the amplitude of the T waves was observed due to the increase in rate, nevertheless in two cases a slight increase in the amplitude was observed without a coincident rise in heart rate. The changes observed were thus not of a major character.

Treadmill exercise. The most consistent and striking changes are evident from the results of this exhausting exercise. In each of the four subjects who performed the test there was less CO_2 removed, although the figures for oxygen consumption were nearly identical in the control and drug periods. The most marked effects were found in the case of D. B. D., in whom the blood sulfanilamide was highest, viz., a drop of 29 per cent in the CO_2 elimination and a change in R.Q. from 1.19 to 0.94. In the case of N. R. it will be seen that there was no change on the first day of sulfanilamide with a blood level of 2.3 mgm. per cent, but a definite change on the second day with a blood sulfanilamide value of 3.6 mgm. per cent. This evidence for difficulty in CO_2 removal was supported by the subjective sense of suffocation which several of the subjects experienced, and by the fact that the hyperpnea during recovery was noticeably more severe and prolonged. Determinations of blood lactate 5 minutes after each run showed similar values before and during sulfanilamide, thus indicating comparable grades of work during the different runs.

The question arises whether decrease in the blood bicarbonate might be the cause of these changes rather than any specific sulfanilamide effect. Accordingly, D. B. D. took 10 grams of ammonium chloride and performed the run 12 hours later. This time his blood bicarbonate (T_{40}) showed approximately the same value as during sulfanilamide. The R.Q. and CO_2 output, however, were definitely higher, although not as high as in the normal state. A similar result is seen in F. C. and J. T. on treadmill runs 24 hours after stopping sulfanilamide. At this time the sulfanilamide had practically disappeared from the blood, but the blood bicarbonate was as low as, or lower than, during the drug administration. It can be seen

that the R.Q. was definitely higher in both instances during this residual acidosis than during sulfanilamide administration.

It can only be concluded from these data that in exhausting exercise the mechanism for CO_2 removal has been definitely impaired in the case of D. B. D. at the time when his blood sulfanilamide content was 4.2 mgm. per cent (the highest level observed in any of the individuals tested) and showed signs of impairment in the other cases.

No significant additions to our evaluation of cardiovascular changes were secured during the severe exercise. The maximum pulse rate was not altered.

Electrocardiograms taken immediately after the run showed small variations, which could only be attributed to changes in heart rate.

TABLE 2

Time in seconds to complete Johnson Code test*

EXPERIMENTAL SUBJECTS	BEFORE SULFANILAMIDE	DURING SULFANILAMIDE	Δ PER CENT	AFTER SULFANILAMIDE
D. B. D.	112.5	127.4	+13.2	113.6
R. C. D.	113.5	122.9	+8.2	102.8
F. M. C.	100.8	113.1	+12.1	90.0
Means	108.9	121.1	+11.6	102.1
CONTROL SUBJECTS	**NO SULFANILAMIDE**	**NO SULFANILAMIDE**	**Δ PER CENT**	**NO SULFANILAMIDE**
S. M. H.	98.9	101.1	+2.1	99.4
D. O. F.	152.8	142.6	−6.7	143.1
Means	125.9	121.4	−2.3	121.2

* An increased time indicates a poorer performance.

Psychological tests. The Johnson Code Test, alone among the three tests used, showed significant impairment during sulfanilamide administration. The scores (time adjusted for errors) are shown on table 2 for the three experimental subjects and two control subjects.

This test is assumed to measure a cortical level of response. On the other hand the Pursuit Meter, measuring at a more nearly reflex level, failed to show more than daily variability in D. B. D. and R. D. In F. M. C. there was slight impairment, probably not significant. Likewise, the form-naming test showed no significant impairment, possibly due to the variability of performance of all subjects on this test.

Practical conclusions as to the effect of ambulatory doses of sulfanilamide in man. The mental and physical handicap of taking the drug in this dosage is greater than the psychological and physiological tests would

indicate. Aside from the vomiting in two of the six cases, each of the subjects had a feeling of considerable malaise and mental incompetence. Such feeling was not conducive to good work in the laboratory during the period of the drug and would undoubtedly impede the skilled activities of workers in industry or the military. In emergency, however, a considerable amount of unskilled labor should still be possible, provided the subjects are free from vomiting.

It should be emphasized that these experiments were made with the drug sulfanilamide and not with any of the newer drugs in the same therapeutic group. It is known that the latter drugs have no *in vitro* effect on carbonic anhydrase. However, the other effects, i.e., on the nervous system, would seem to be similar and to vary only in magnitude. It remains to be determined whether any of these drugs may be administered in adequate therapeutic dosage and still allow the patient to carry on safely a fully active regime. However, the low cost of the simpler sulfanilamide will probably continue to dictate its use whenever possible. The toxic effects noted above in adults do not necessarily contradict the benign effects reported in rheumatic children (8) given the drug in 2 gram daily doses as a preventive against recurrent attacks of rheumatic fever. In the first place, it has been a common clinical observation that children tolerate the drug with less apparent toxic effects. Furthermore, almost all the children so treated have been severely restricted in their physical activity due to heart damage.

SECTION II. THE RELATION BETWEEN SULFANILAMIDE, CARBONIC ANHYDRASE IN THE INTACT RED CELL AND THE RATE OF CO_2 OUTPUT IN VIVO. Mann and Keilin (4) have shown that in dilute solutions of carbonic anhydrase the activity of the enzyme, as measured by the magnification it produces of the rate of CO_2 output from M/10 phosphate-M/10 bicarbonate mixtures at pH 6.8 (Meldrum and Roughton (9)), is reduced to one-half by 2×10^{-6} M sulfanilamide, i.e., by 0.035 mgm. per cent. At the blood level attained by the subjects in table 1, viz. 3 to 4 mgm. per cent, the activity should be reduced 100-fold, if, as Davenport's recent data (10) suggest, 1 molecule of sulfanilamide inhibitor combines reversibly with 1 molecule of enzyme, i.e., $E + I \leftrightharpoons EI$.

Roughton (11) has calculated (I) that the rate of the reaction $H_2CO_3 \rightleftharpoons CO_2 + H_2O$ needs to be magnified 150-fold within the blood, if the CO_2 in the lung capillaries is to come to within 10 per cent of equilibrium with the CO_2 in the alveolar air in a time of one second, or 75-fold if the average time spent by the blood in the lung capillaries is two seconds. The figure of one second is probably a minimum, whereas that of two seconds is probably not far from the average. These calculations apply both to rest and to moderate work.

(II) Carbonic anhydrase at the concentration present in the red cell

would magnify the rate of CO_2 output from the Meldrum-Roughton buffer mixture (v. supra) 1500-fold at 38°C. if the activity of the enzyme remains proportional to the concentration up to these very high concentrations of the latter. This figure of 1500 is increased to 2700 when the higher average anhydrase content of human blood, subsequently found by Hodgson (12), is used.

In the red cell in vivo the substrate concentration is lower and the pH more alkaline than in the Meldrum-Roughton solution. Both these factors would, according to unpublished data by Roughton and Booth, increase the magnifying effect of the enzyme, the former about 2-fold and the latter about 1.4-fold, thus leading to an over-all increase from a magnification of 2700 to one of 7500. The M/10 phosphate in the Meldrum-Roughton solution and the chloride content of the red cell are both inhibitory to about the same extent at pH 7.1 so that no correction is needed for the different salt contents of the two media. There is, however, an additional factor which should increase the figure of 7500 still further. This is the activating effect of the $—NH_2$ containing compounds (Leiner, 13) present in the red cell.

Even, however, if we accept the minimal figure of 7500 as the magnification produced by the anhydrase of the red cell and assume that 3 to 4 mgm. per cent sulfanilamide is able to reduce it 100-fold,[1] we are still left with a magnifying factor of 75, which according to Roughton's calculation (I) might be enough to account for the rate of CO_2 output at rest and in moderate work. On this basis, the maintained rate of CO_2 output under the conditions of table 1 (except for the exhausting exercise) is explained.

These arguments, though fairly convincing, would be much strengthened by direct observations on the activity of the carbonic anhydrase in the intact red cell. In absence of inhibitors the reactions in the red cell are too fast to be followed by manometric methods and can only be recorded by the rapid-flow methods of Hartridge and Roughton (14, 14a); but the interpretation of the results is seriously complicated by non-enzymic factors, such as the rate of diffusion of CO_2 through the membrane and body of the red cell. In the presence of sulfanilamide, however, the rate of CO_2 uptake by CO_2-free red cell suspensions shaken with air containing 5 per cent CO_2 is slow enough to be followed by the manometric technique, described by Meldrum and Roughton (15) and used subsequently by Booth (16). Since the results and conclusions so obtained are but rough, they will therefore only be described briefly.

The procedure was as follows. Human blood (with oxalate or heparin as anticoagulant) was centrifuged and the plasma replaced by 0.9 per cent

[1] Davenport (10) has recently shown that the inhibitory effect of sulfanilamide progressively falls with increase in enzyme concentration and finally reaches a value only about $\frac{1}{7}$ of that found by Mann and Keilin in dilute enzyme solutions.

NaCl. The cell suspension was then freed of nearly all its CO_2 by repeated shaking in vacuo at 37°C., and then recentrifuged. One cubic centimeter of cells was mixed in a 40 cc. boat with 2 cc. of 0.9 per cent NaCl containing concentrations of sulfanilamide varying from 0.03 to 0.5 per cent. The boat was placed in a shaker at 0°C. or room temperature (22°C.) and the air in the boat displaced by passing a gas mixture containing 5 per cent CO_2, 25 per cent O_2 and 70 per cent N_2 through it for two minutes. After 2 to 3 minutes for temperature equilibriums, shaking was started and the CO_2 uptake followed by the gauge readings of the manometer.

At 0 to 3 mgm. per cent sulfanilamide the observed rates are practically constant, being conditioned not by the chemical reaction rates in the suspension but by the rate of diffusion of CO_2 from the gas to the liquid phase; but at sulfanilamide concentrations of 20 mgm. per cent the observed rates are appreciably lower, and in fact depend both on the enzymic rate and the diffusion factor. Separation of the enzymic rate can, however, be made very roughly by the method to be described by Roughton in a forthcoming paper. On this basis we find that the time required for 90 per cent of the CO_2 uptake to be completed as regards the enzymic process (as distinct from the diffusion process) is about 110 seconds at 0°C. and 22 seconds at 22°C. for a sulfanilamide concentration of 20 mgm. per cent. Independent data by Davenport (10) on dog red cells agree to within 10 to 20 per cent of these figures. At 3 to 4 mgm. per cent sulfanilamide the corresponding times should be about 20 seconds at 0°C., 4 seconds at 22°C. and (by extrapolation) 1.4 seconds at 38°C., if Davenport's evidence that one molecule of inhibitor combines reversibly with one molecule of enzyme is correct. Since the enzyme magnifies both CO_2 uptake and output rates equally, a time of 1 to 2 seconds should also be required to reach within 10 per cent of equilibrium as regards CO_2 output.

These more direct experiments on the red cell suspensions thus lead to the same conclusions as the previous work on dilute enzyme solutions. It is hoped that it may later be possible, by the Hartridge-Roughton rapid-flow methods, to make direct observations of the rate of CO_2 output from red cells poisoned with only 3 to 4 mgm. per cent sulfanilamide, and thereby check directly whether the residual activity of the carbonic anhydrase in the red cells under these conditions is sufficient, or whether the maintenance of the rate of CO_2 output in table 1 might be in part occasioned by enhanced function of the carbamino mode of CO_2 transport. Normally, according to Ferguson and Roughton (17) and Ferguson (18), one-quarter to one-third of the CO_2 rapidly transported by the blood is brought about by rapid reversible combination between CO_2 and $—NH_2$ groups in the hemoglobin molecule, a reaction which does not require the functioning of carbonic anhydrase.

An attempt was made in the subjects at rest and performing moderate exercise to detect changes in the equilibrium pressures of CO_2 in the blood and alveolar spaces. Such measurements, it was hoped, might give some hint of conditions more favorable for the carbamino transport of CO_2. The measurements made were 1, the alveolar pCO_2; 2, the arterial blood pCO_2, and 3, the "virtual mixed venous pCO_2." The last value was measured by rebreathing a suitable CO_2-O_2 mixture for a period shorter than the blood recirculation time, and presumably measures the pCO_2 of the mixed venous blood which has been oxygenated but has neither lost nor gained CO_2. Several unknown factors (possibly changing cardiac output, the O_2 saturation of the mixed venous blood, the possible failure of CO_2 equilibrium during oxygenation of the venous blood) make definite conclusions from analysis of these data impossible. However, we present two trends observed in our data, since they indicate possible adjustments made in vivo to inhibition of carbonic anhydrase, and may point the way to future better controlled experiments. These trends were 1, an increase in the *difference* between the CO_2 tensions in the arterial blood and the mixed venous blood during moderate exercise after sulfanilamide. This condition, other things being equal, would favor an increase in the proportion of CO_2 transported in the carbamino form; 2, also, during moderate exercise the pCO_2 of the arterial blood (after withdrawal from the body) relative to that of the alveolar air tended to be higher during sulfanilamide than before taking the drug. This might indicate insufficient activity of the carbonic anhydrase, since in that case the free CO_2 of the blood might be readily removed down to a low pressure from the blood in the lungs before equilibrium in the reaction $H_2CO_3 \rightleftharpoons H_2O + CO_2$ is reached. The attainment of this equilibrium after the blood had left the lungs would lead to a subsequent increase in the pCO_2 of the arterial blood at the expense of its bicarbonate content.

Thus, whereas the best calculations from in vitro tests would seem to indicate just sufficient residual activity of the enzyme for moderate work in the presence of 3 to 4 mgm. per cent of sulfanilamide, there is suggestive evidence from the actual subjects that in vivo adjustments have begun to appear.

CONCLUSIONS

1. At rest and in moderate exercise (5–6 times the resting metabolism) the rate of CO_2 elimination is unchanged in human subjects by administration of sulfanilamide to a blood concentration of 3 to 4 mgm. per cent.

2. In exhausting exercise there is definitely some handicap in CO_2 removal, resulting in the damming back of CO_2. This adds to the acidosis of lactate formation and results in prolonged dyspnea during recovery.

3. The degree of psychological and general physical handicap observed

in subjects taking two to three grams of sulfanilamide daily was severe enough to make its prophylactic and therapeutic use unsuitable in patients expected to continue exacting or strenuous work, especially if this requires skill.

4. According to calculations for moderate exercise normal CO_2 exchange in the lungs requires sufficient carbonic anhydrase in the red cells to speed the reaction $H_2CO_3 \leftrightharpoons H_2O + CO_2$ 75-fold. Independent deductions from previous studies of the inhibitory action of sulfanilamide on dilute enzyme solutions and from present measurements of the enzyme inhibition in the intact red cell suggest that the residual enzyme activity in the presence of 3 to 4 mgm. per cent sulfanilamide is at or near the above threshold.

5. There is however some evidence from the measurement of carbon dioxide tensions in the alveoli and in the venous and arterial blood of subjects performing moderate exercise after sulfanilamide that the enzyme activity of the carbonic anhydrase is not quite adequate and that the carbamino mechanism may be performing a greater share in the transport of CO_2.

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THE EFFECTS OF RENIN AND OF ANGIOTONIN ON THE RENAL BLOOD FLOW AND BLOOD PRESSURE OF THE DOG

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The effect of intravenous injection of renin on the renal circulation has been observed by a variety of methods and, with one exception, the characteristic response has been shown to be a decrease of renal blood flow. Steele and Schroeder (1), using a thermostromuhr, observed the action of renin on renal blood flow of dogs during recovery from anesthesia (pentobarbital sodium) and found that renin either failed to alter renal blood flow or caused a slight increase. Under the conditions of their experiments the renal circulation responded characteristically to epinephrine. Their findings were not in agreement with those of previous workers (2–5) nor with those of one of us (Corcoran) and Page (6) who found a significant decrease of renal blood flow in trained dogs during intravenous infusions of renin. Because of this difference in results it seemed advisable to make further observations on the effect of renin on renal blood flow by means of a thermostromuhr and under a variety of experimental conditions. Data also are presented in this report concerning the action of angiotonin, the vasoconstrictor formed in the interaction of renin and renin activator (7).

METHOD. The direct current thermostromuhr as described by Baldes and one of us (Herrick, 8) was employed for all measurements of blood flow. Blood pressure was recorded optically by means of a glass spoon manometer (9). Seven dogs were used for this study. Four were anesthetized with pentobarbital sodium in acute experiments. The remainder of the experiments, in which observations were extended over several days, were done on trained dogs. The dogs weighed between 10 and 16 kgm. except for one which weighed 5.9 kgm. The operative procedures for the acute experiments were as follows: Through either a midline or a lumbar incision parallel to the spine the renal artery was exposed and carefully freed from connective tissue. After a thermostromuhr unit of proper size had been applied, the wound was closed. Following this, either the femoral or the carotid artery was cannulated and connected to

the glass spoon manometer for the measurement of blood pressure. In two acute experiments blood flow was also measured in the femoral artery. In the experiments on the trained dog the unit was applied to the renal artery through a lumbar incision parallel to the spine, ether anesthesia and sterile technic being employed. Observations of blood flow were not begun until the dog had recovered from the immediate effects of the operation. At the time of measurement of blood flow the mean femoral blood pressure was obtained by insertion of a hypodermic needle into the artery after connection with a glass spoon manometer. If, for any reason, cannulation of the artery was preferred for the measurement of blood pressure, the

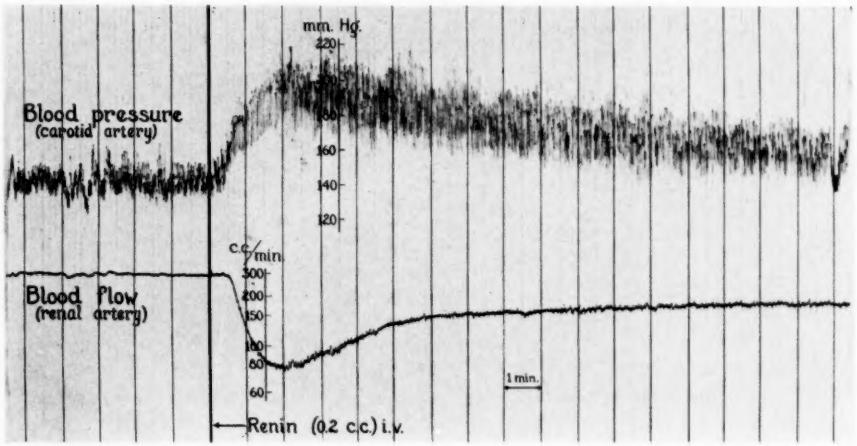

Fig. 1. Effect of renin on the blood flow in the renal artery and carotid arterial blood pressure.

necessary operative procedure was performed with the animal under local infiltration of pontocaine hydrochloride and sterile technic was employed.

After satisfactory control observations either renin or angiotonin was injected intravenously. Usually an intravenous infusion, at a relatively slow rate, immediately followed the initial intravenous injection.

Results. Renin and angiotonin produced the same consistently significant effect on the flow in the renal artery, that is, a marked decrease (figs. 1, 2 and 3). Angiotonin caused a more immediate and more transient effect than renin. This characteristic difference between the effects of renin and angiotonin has been reported previously (10). The two substances produced similar effects on the flow of blood in the femoral artery, that is, a transient decrease followed by a somewhat more prolonged increase (fig. 3).

The blood pressure increased significantly after injections of either renin or angiotonin.

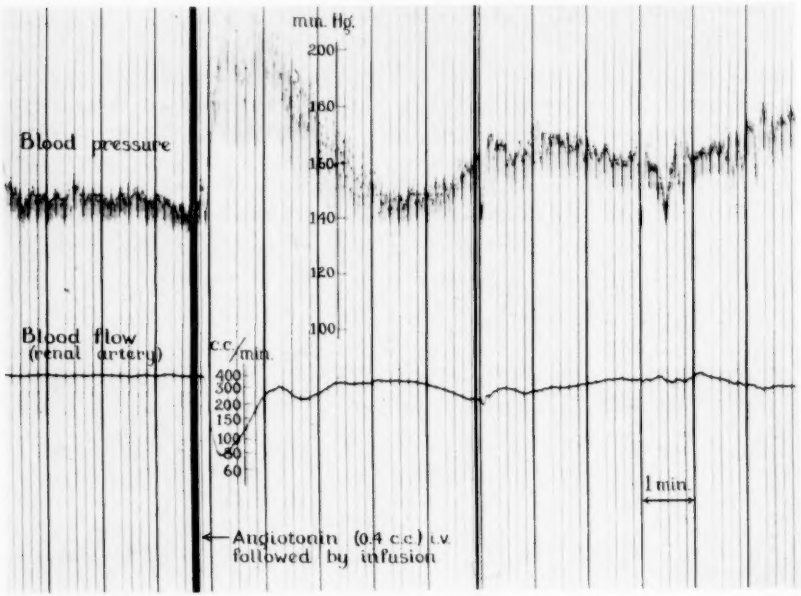

Fig. 2. Effect of an intravenous injection of angiotonin followed immediately by infusion. Infusion rate: 0.21 cc. per minute for twenty-seven minutes.

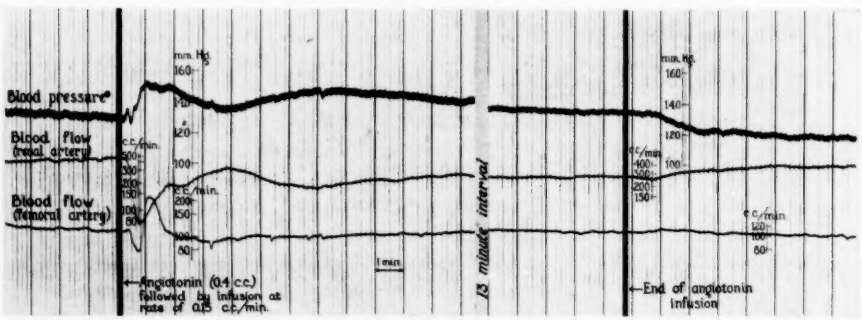

Fig. 3. Effect of angiotonin on the blood flow in the renal and femoral arteries and on the femoral arterial blood pressure. Note the changes following the end of infusion. The dose of 0.4 cc. was given intravenously and was followed immediately by infusion.

When a second injection of either substance was made within a few minutes after the first, usually no effect or a diminished effect was observed. Renin may make the dog refractory to angiotonin as well as to renin and likewise angiotonin may make the animal refractory to renin as well as to itself. If a sufficient time elapses, the effect of the first injection may be obtained again. This phenomenon has been described previously (11). One of the dogs failed to show any tachyphylaxis, in that succeeding injections within a few minutes of each other caused the same marked reduction in renal blood flow and increase of arterial pressure.

No significant difference could be detected in the effects of either angiotonin or renin as between trained dogs and dogs under pentobarbital sodium anesthesia (table 1). This observation in dogs is contrary to that reported for renin by Pickering and Prinzmetal (12) in the rabbit, but it confirms

TABLE 1

Comparison of the effects of renin and of angiotonin administered intravenously with and without anesthesia

EXPERIMENT	WEIGHT OF DOG	ANESTHETIC AGENT	PERCENTAGE DECREASE IN BLOOD FLOW	
			Renin (dose)	Angiotonin (dose)
	kgm.			
1	16.5	Pentobarbital sodium	64 (0.2 cc.)	63 (0.9 cc.)
2	10.5	None	66 (0.4 cc.)	76 (0.4 cc.)
3	14.5	None	70 (0.4 cc.)	79 (0.4 cc.)
4	12.5	Pentobarbital sodium	40 (0.2 cc.)	82 (0.4 cc.)
5	5.9	None	90 (0.3 cc.)	94 (0.5 cc.)
6	13.2	Pentobarbital sodium	60 (0.4 cc.)	

that of Wakerlin and Chobot (13) with regard to the effect of anesthesia on the action of renin in dogs.

Kohlstaedt and Page in unpublished observations have shown that the isolated perfused kidney of the dog responds to injections of renin or angiotonin by vasodilatation when the kidney has been subjected to prolonged ischemia during or after transfer to the Dale-Schuster pump-lung circuit. On the other hand, when the kidney is transferred rapidly into a circuit from which the vasoconstrictor of defibrinated blood has been removed by lung perfusion, it responds to injections or infusions of renin or angiotonin by sharp vasoconstriction. In either case, the response of the isolated kidney to injections of epinephrine, tyramine and pitressin is the same. The observations on the isolated perfused kidney of the dog under adverse conditions parallel those of Steele and Schroeder on dogs recovering from anesthesia. The difference between their results with

renin and those here and elsewhere reported therefore may represent a difference in experimental conditions which adversely affects renal circulation in its response to renin.

CONCLUSIONS

Both renin and angiotonin decrease the blood flow in the renal artery and increase arterial pressure. In a few observations on the flow in the femoral artery it was found that both renin and angiotonin caused a diphasic effect consisting of an initial transient decrease followed by a somewhat more pronounced and prolonged increase. Pentobarbital sodium anesthesia did not seem to alter the hemodynamic effects of these substances.

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PERMEABILITY OF ERYTHROCYTES TO RADIOACTIVE POTASSIUM

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The experiments to be reported consist of two sorts: 1, the injection of radioactive potassium into animals and the measurement of its erythrocyte penetration in vivo, and 2, the addition of radioactive potassium to the plasma of human blood kept in vitro at 37°C. by the use of an anticoagulant. Preliminary reports of this work have already been made (Mullins et al., 1941) and the pertinent literature has been quoted (Dean et al., 1941).

To measure penetration it is necessary to analyze for total potassium (K^+) in both cells and plasma and to determine the simultaneous concentration of radioactive potassium (K^*) by an immersion type of Geiger-Müller counter. The ratio of these two quantities (K^*/K^+) may be called the potassium radioactivity (KRA).[1] The percentage penetration is then $100 \times KRA_{cells}/KRA_{plasma}$. Thus penetration is said to be complete when the isotopic composition of the potassium inside the cell has become equal to that outside by exchange of K^+ for K^*.

METHODS. Details of our methods of preparing the radioactive samples of KCl for injection have been previously described (Noonan et al., 1941). The material was injected usually subcutaneously or in the dorsal lymph sac for frogs. Cats were anesthetized with ether and bled from the heart or the carotid artery. Rabbits were bled by heart puncture or from an ear vein. Rats were killed by stunning and blood was collected by cutting the throat. Clotting was prevented by heparin or oxalate. Frogs were bled by syringe from the aorta after being immobilized by crushing the brain.

RESULTS. Figure 1 shows the results of the in vivo experiments with several different animals. The experiments with rats have been previously reported (Dean et al., 1941) so that the experimental points are omitted. In general each point represents a separate animal except in the case of the rabbits where several samples of blood can be taken at intervals from

[1] 100 divided by (counts injected per kilogram body weight) and multiplied by (counts per milliequivalent of potassium in sample).

an ear vein. Since each point represents a separate animal, considerable variation is to be expected but nevertheless the rabbit data in particular fall along a very smooth curve, the penetration being 50 per cent complete in about 20 hours. A marked species difference is evident. The penetration of radioactive K into dog cells is shown in figure 2. Most of the points were obtained from a single in vitro experiment in which the cells were equilibrated in their own oxalated plasma at 37°C. with continuous rotation and were sampled at intervals. At the beginning of the experiment 0.5 ml. of 2.2 per cent radioactive KCl was added to 30 ml. of freshly drawn blood. This provides a much higher count in the plasma than can be obtained in experiments in vivo. The counts are correspondingly more accurate particularly in the cells where the low total K tends to give a

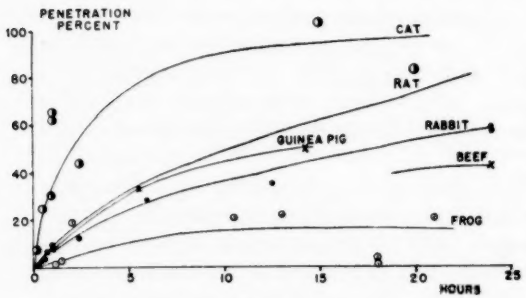

Fig. 1

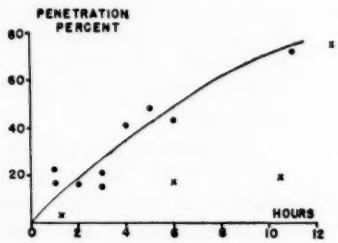

Fig. 2

Fig. 1. Values of 100 × relative *KRA* for erythrocytes of different species plotted against time. Experimental points for the rat are given in a previous report (Noonan et al., 1941).

Fig. 2. Values of relative *KRA* × 100 for dog erythrocytes. Dots represent in vitro observations while crosses are from 3 different experiments in vivo in which the cell counts were too low for satisfactory accuracy.

low count. In addition to the single in vitro experiment three experiments on dogs were tried in vivo, the K* being injected subcutaneously and blood being taken at intervals by venipuncture. The results are indicated in figure 2 by crosses but the low count in the cells makes the results hardly accurate enough to report. Since the higher points as it happens are the more accurate we do not consider that the data demonstrate any less rapid penetration in vivo than in vitro.

In figure 3 are plotted our data for human red cells. The dots represent the in vitro experiments while the circles represent those obtained in vivo. We have already reported one somewhat similar in vitro experiment which is not included in figure 3 (Dean et al., 1941). It seemed important to extend the data, however, because the cells in these early experiments were not in plasma and there was danger that the permeability might have

been abnormally great. Actually the rate of penetration turned out to be somewhat greater than in the early preliminary experiments.

The procedure in these experiments has been varied widely in order to see whether the penetration of K was due to damage from handling. As anticoagulants we have used heparin, oxalate, citrate, and chlorazol fast pink without being able to establish any uniform difference. In defibrinated blood, however, the penetration did appear to be significantly accelerated. In one experiment we endeavored to avoid the use of any anticoagulant by drawing the blood in paraffined vessels and keeping it chilled until after centrifugation. Another sample of blood was drawn with heparin and kept chilled as a control. Only short experiments were possible by this technique but the results showed as much penetration in 45 minutes as would be expected from the graph of figure 3, and no differences with or without heparin were apparent. We have also varied the gas mixture

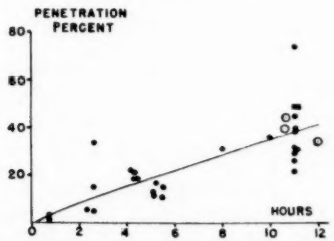

Fig. 3. Values of relative $KRA \times 100$ for human cells. Experiments in vitro (dots) and in vivo (circles).

with which the blood was equilibrated using oxygen, pure nitrogen, 30 per cent CO_2 in oxygen, and pure carbon monoxide. Again we were unable to obtain significant differences in penetration rate although the variations were large. All these experiments with different anticoagulants and different gases are plotted indiscriminately in figure 3 as proof that exchange of K^* for K^+ does proceed at a more or less definite rate.

In most of these experiments we have simply centrifuged the blood sample in a graduated tube, removed the plasma as cleanly as possible and analyzed both cells and plasma for both K^+ and K^*. In some experiments, however, we have first washed the cells once in isotonic sucrose or isotonic NaCl without appreciably affecting the results. In some cases, instead of separating, washing, and then counting cells we have analyzed plasma and whole blood and then have calculated the K^+ and K^* inside the cells from these figures and the hematocrit value. This also gave no significantly different results. Since our earlier experiments with Ringer's solution gave slightly less penetration than we now observe we tried some

further experiments with cells suspended in Ringer's solution but the points obtained fell closely on the curve given in figure 3. We must conclude therefore that the rate of exchange of potassium across the red cell membrane is fairly constant and is not subject to appreciable variations from different methods of handling.

Special mention should be made of the three points in figure 3 representing experiments done in vivo in man. These offer considerable difficulty because the number of counts obtainable in a single sample of KCl is limited and in an animal as large as man it does not suffice to give a count in the plasma which is much larger than the background count. To obtain a sufficiently high count as large a sample of K*Cl as possible was ingested promptly after its preparation. A large sample of blood (50 ml.) was drawn 12 hours later. After centrifugation cells and plasma were separately ashed in nitric acid and superoxol and concentrated by evaporation to a

TABLE 1

Penetration into human cells in vivo

SUBJECT	KCl INGESTED		TIME	POTASSIUM		COUNTS/LITER		COUNTS/K		PENETRATION
				Cells	Plasma	Cells	Plasma	Cells	Plasma	
	mgm.	*counts per min. × 10^{-6}*	*hrs.*	*m.eq./l.*	*m.eq./l.*					*per cent*
L. J. M.	150		10.8	73.7	3.46	858	91.4	11.6	26.4	44
T. R. N.	288	2.8	10.8	84.5	3.19	909	86.6	10.8	27.1	40
W. O. F.	218	8.0	12.0	57.7	4.01	233	47.4	4.04	11.8	34

Counts per liter are recorded in arbitrary numbers. The counts per minute in the ingested KCl were approximate, no allowance being made for the counts not recorded because of the inefficiency of the counter.

volume of about 4 or 5 ml., samples of which were counted and analyzed. The data from these three experiments constitute the most convincing proof of the permeability of human red cells to potassium and the data are given in table 1. It is striking that the results fall precisely on the curve obtained from the many in vitro experiments which we have performed. This is additional evidence that the permeability of the cells is not modified in any detectable way by handling the cells in vitro. Even in experiments where the cells in vitro were slowly hemolyzing the penetration into the cells which were still intact seemed to be in no way accelerated.

In table 2 a comparison has been made between the rates of penetration into the red cells of various animals. Figures for the diameters of the cells and their potassium contents are included. To understand this comparison some theoretical discussion is necessary.

Consider a red cell of volume V_1 ml. and surface area S cm^2 and containing K_1 m. mols. of potassium per ml. This cell is suspended in a medium

of volume V_0 containing potassium in a concentration K_0 mM per ml. The cell is assumed to be at equilibrium as to potassium so that equal numbers of K ions pass both in and out. Let p = the number of millimols of K which pass in one direction per cm² of surface per hour. Let x_0 and x_1 = the ratio of radioactive to total K or $\left[\frac{K^*}{K^+}\right]$ outside and inside respectively. Then $p\,x_0 S$ = mM of radioactive K* which enter the cell

TABLE 2

Permeability of erythrocytes to potassium

	VOLUME $V \times 10^{-12}$	SURFACE $S \times 10^{-8}$	POTASSIUM $K_1 \times 10^3$	k	PERMEABILITY $p = \frac{K_1 k V}{S}$ (FROM k)	TIME FOR 30 PER CENT EXCHANGE t	PERMEABILITY $p = \frac{0.35 K_1 V}{tS}$ (from t)	TURNOVER $\frac{100\ pS}{K_1 V}$
	μ^3	μ^2	$mM/l.$	$hours^{-1}$		*hours*		*per cent/ hour*
Dog	57	96	7.7	0.107	0.49	3.4	0.47	10.3
Cat	30	62	12.0	0.25	1.45	0.8	2.6	45
Rabbit	54	93	90	0.041	2.1	7.0	2.6	5.0
Man	78	119	85	0.045	2.5	8.2	2.4	4.3
Guinea pig	58	96	89	0.06	3.2	4.5	4.2	7.8
Rat	48	86	90	0.107	5.4	4.5	4.0	8.0
Frog	4015	942	76	0.016	5.3	16*	4.5	1.4

* Taken as twice the time for 15 per cent exchange. The permeability, p, is in units of mM of K per cm.² per hour $\times 10^7$. Figures for V and S were taken from Ponder (1924) except those for the frog which were calculated on the assumption that the cell is a prolate spheroid with major and minor axes of 25.4 and 17.4μ respectively. The hourly turnover of K is taken from values of p calculated from t.

per hour while $p\,x_1\,S$ = the mM of K* which leave in the same time and the rate of change of x_1 is

$$\frac{dx_1}{dt} = pS\,\frac{(x_0 - x_1)}{K_1 V_1} \tag{1}$$

If a is the initial value of x_0 then

$$x_0 = (a - x_1)\,\frac{K_1 V_1}{K_0 V_0} \tag{2}$$

Substituting this in equation 1 and integrating we obtain

$$\log_e\left[a - x_1\left(1 + \frac{K_1 V_1}{K_0 V_0}\right)\right] = -\left[\frac{pS}{K_1 V_1}\left(1 + \frac{K_1 V_1}{K_0 V_0}\right)\right]t \tag{3}$$[2]

If the cell is suspended in a medium of large volume so that x_0 is constant or if x_0 is kept constant as it is approximately in the experiments in vivo

[2] We are indebted to Dr. H. A. Blair for this form of the equation.

by exchange with the rest of the body then $V_0 = \infty$ and equation 3 can be simplified to the form

$$\log_e (1 - x_1) = - \frac{pSt}{K_1 V_1} = - kt \tag{4}$$

where k is the slope of the graph obtained by plotting $- \log_e (1 - x_1)$ against t. Then

$$p = \frac{kK_1 V_1}{S} \tag{5}$$

The values of k so obtained are given in table 2. These figures are fairly well defined by this method for dog, rat and rabbit. The points for guinea pig and man are few but reliable; for the frog and the cat the logarithmic plot is very poorly defined and these values of k are therefore subject to large error. From these values of k the permeability, p, has been calculated by equation 5. The results show permeabilities varying from 0.49 for the dog to 5.3 for the frog.

Similar values may be obtained by another method of analysis whereby the times, t, for 30 per cent exchange are derived graphically from graphs in figure 1. These values of t are given in table 2. The average rate of change of x_1 during this time is $\frac{0.3}{t}$. If this average rate is assumed to be obtained at the middle of this period or when $(1 - x_1) = 0.85$ then the permeability, p may be calculated by putting equation (1) equal to $\frac{0.3}{t}$ and solving for p, taking $(1 - x_1) = 0.85$. The values of p so calculated are given in table 2. They agree fairly well with similar values calculated from k with the exception of the cat where the experimental points were so widely varied.

From these figures for p it is possible to calculate the rate with which the K inside any one of the red cells exchanges with K in the serum. The dimensions of p are millimols of K exchanged per square centimeter of surface per hour. Therefore, the total exchange in any one of the red cells is pS and the total amount of K in the cell is $K_1 V_1$. The hourly turnover in per cent is therefore, $100 \frac{pS}{K_1 V_1}$. Figures so obtained are given in the last column of table 2. All the cells exchange 4 to 10 per cent of their total K in the course of one hour with the exception of cat and frog. The cat cell exchanges 45 per cent of its K, this large figure being due to its small K content and large specific surface. The small figure for the frog (1.4 per cent) is due to its very large size and consequently its small specific surface. Of all the cells studied the dog cells are the least permeable; indeed it might be stated that all cells are of approximately equal

permeability with the exception of the dog. In spite of this low permeability the total K content is so small that the dog cells are still able to exchange a normal fraction of their total K (10.3 per cent) in one hour. The rapid exchanges of K in dog erythrocytes were also noted by Cohn (1940).

From a graph for frog cells shown in figure 1, it might be concluded that complete exchange never occurs in this species. Nevertheless the calculated permeability constants are not abnormally low. It is possible, therefore, that the apparent failure to obtain complete exchange in the 24 hours available for experimentation is due entirely to the large size of these cells and their small surface mass ratio. It may also be that only a part of the cell potassium, perhaps that in the cytoplasm, is available for exchange.

In making this analysis it has been assumed that x_0 is constant and equal to 1. This assumption may be avoided in analyzing the in vitro dog cell experiment since the values of V_0 and V_1 are known and may be substituted in equation 3. The result gives a value of 0.42 instead of 0.49 for k indicating that the error involved was not serious.

In the case of the experiments done in vivo there may be some question as to the propriety of assuming that x_0 is constant. After intravenous or intra-arterial injection of K* the value of x_0 is enormously high immediately after the injection but it falls very rapidly in a few minutes. After intraperitoneal or subcutaneous injection K* is disposed of to the tissues almost as fast as it is absorbed into the blood so that x_0 is fairly constant. The values of KRA $(= x_0)$ for plasma calculated from the rabbit data of table 2 for times <2.5 hours average 2.8 while the average value for times >10 hours was 2.4. A curve previously published for rabbits (Dean et al., 1941) and recalculated shows somewhat higher values for times <3 hours but the diffusion coefficient calculated from this experiment by graphical integration, i.e., allowing for actual values of x_0 was 0.665×10^{-3} min.$^{-1}$ or 0.04 hr.$^{-1}$ as compared to 0.041 for rabbits in table 2. Our earlier figure for rats was 0.06 hr.$^{-1}$ instead of 0.107 by the newer analysis of the same data in table 2 but this difference is mostly due to the way in which the curve for cells was drawn through the scattered points and the newer value is at least as good as the earlier one. The newer figure in table 2 for human cells is 3.5 times as large as the earlier one but it is based on many more and better experiments and the difference is not due to the method of analysis. Because of the scatter of points obtained, each from a separate animal, the data hardly justify more rigid analysis and the results are approximate only.

In our previous paper we showed that rat red cells were permeable to K but suggested the possibility that they might be impermeable to Na. Otherwise it would be difficult to understand why Na did not exchange for

K. Now, however, we have shown that dog cells are permeable to K while Cohn and Cohn (1939) have previously demonstrated a penetration of Na. It remains to show that cat cells are also permeable to both K and Na. The data available concerning the penetration of radioactive Na into cat cells are presented in table 3. Ten milliliters of blood were taken from an anesthetized cat with heparin and added to 0.1 ml. of 0.85 per cent radioactive sodium chloride. The mixture was rotated at 37°C. and sampled after 1 and 4 hours. Radioactive sodium chloride was also injected into the cat intraperitoneally and a blood sample was taken 3 hours later. After centrifugation, cells and plasma were separated, digested in HNO_3 and analyzed for Na and radioactivity as usual. The cells were washed once in two volumes of saline before they were analyzed. The results leave no doubt that penetration of Na has occurred to the extent of at least 10 per cent in about 3 hours.

TABLE 3

Permeability of cat cells to Na in vitro (heparinized blood)

	PER CENT CELLS	Na CONTENT		CORRECTED COUNTS PER GRAM		COUNTS PER M.EQ. OF Na		PENETRATION
		Cells	Plasma	Cells	Plasma	Cells	Plasma	
		m.eq./l.	*m.eq./l.*					*per cent*
1 hour	38	96.6	131.5	20	225	0.21	1.71	13.1
4 hours	45	82	151	51.5	227	0.63	1.50	42
3.4 hours in vivo	39	(89)*	(141)*	16	192	0.18	1.36	13.2

* Average figures from the other 2 samples.

It must be concluded from these results that the classical cation impermeability of red cells is only relative. The membrane is undoubtedly much more permeable to anions than to cations but cations do penetrate and the differences in concentration of potassium which are found between the inside and outside of erythrocytes must be due to something other than impermeability to potassium.

Hevesy and Hahn (1941) in their latest report have found that "in experiments lasting a few days an appreciable part of the corpuscle potassium of the rabbit, thus a much larger part than found by us previously, is replaced by plasma potassium." Even so the exchange which they report is far less than we have found. Unless their rabbits differed from ours we can only suggest again (Dean et al., 1941) that there may have been a slight contamination with radioactive Na.

Kurnick (1941) has recently concluded that human erythrocytes are normally impermeable to K and has erroneously quoted us in support of this conclusion. It should be pointed out, however, that his experiments

show only that the total amount of K in the cells does not change under normal conditions and show nothing about their permeability.

The results with K penetration of erythrocytes reported by Danowski (1941) tend to show that in the human red cell, K transfer from cells to plasma and from plasma to cells can be demonstrated under certain conditions connected with metabolism although it is to be emphasized that such studies do not permit an evaluation of the normal K permeability constant.

SUMMARY

The permeability of erythrocytes to potassium is confirmed by the use of radioactive potassium in cells of man, dog, rat, cat, guinea pig and frog. The results are calculated in millimols of potassium transferred simultaneously in both directions per hour. Allowance is made for differences in volume and surface area of the various cells. Human erythrocytes show 40 per cent penetration in 12 hours in vivo and this rate is unchanged in experiments in vitro. Dog erythrocytes are least permeable and frog cells the most permeable. Cat erythrocytes exchange 45 per cent of their potassium per hour, frog cells only 1.4 per cent while other cells exchange 4 to 10 per cent. Cat erythrocytes are shown to be permeable to radioactive Na so that both cat and dog cells have now been shown to be permeable to both Na and K.

We are indebted to Dr. Dubridge and the cyclotron team of the Physics Department for our supply of radioactive potassium and to Dr. W. F. Bale of the Radiology Department for advice regarding the operation of the counter.

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A DIABETES INSIPIDUS-LIKE CONDITION PRODUCED BY SMALL DOSES OF DESOXYCORTICOSTERONE ACETATE IN DOGS

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The administration of desoxycorticosterone acetate (DCA[1]) to normal dogs in daily doses of 20 to 25 mgm. has resulted in the production of a syndrome of polydipsia and polyuria, resembling diabetes insipidus (DI) (3) (4). The addition of sodium chloride to the drinking water of these animals increased the excessive fluid exchange, but it was noted that pitressin was relatively ineffective and fluid restriction did not lead to dehydration (4). A curious periodic weakness, "with inability to stand or raise the head", was present when the "diabetes insipidus" was most apparent and the effects produced by these large doses were viewed as toxic (3). It seemed of value, therefore, to study the effect of smaller doses of DCA in normal dogs. We chose a daily dose of 2 mgm. per animal because this is about the amount of hormone which will maintain an adrenalectomized dog in satisfactory electrolyte balance (10). The effect of a larger dose (4 mgm.) was also studied for comparison.

Our observations were made on 5 female dogs weighing 8.5 to 12 kgm. The animals were kept in individual metabolism cages and fed a meat diet (25 to 35 per cent fat) supplemented with brewer's yeast, cod liver oil, bone ash, and 2 or 4 grams of sodium chloride. The intake of water and output of urine were recorded. The dogs were catheterized at the end of each 24 hour period, and the chloride content of the 24 hour urine specimen was determined daily. After a preliminary period of observation of several months, 3 of the dogs received 2 mgm. of DCA in 0.8 cc. of sesame oil subcutaneously each day. Two dogs received daily injections of 4 mgm. of the hormone. In addition to measuring the fluid exchange, we studied the effect of fluid restriction, the diuretic responses to a standard dose of distilled water and to a 1 per cent saline solution (25 cc. per kgm.)

[1] Most of the desoxycorticosterone acetate used in these experiments was kindly furnished by Ciba Pharmaceutical Products, Inc. Additional amounts of the compound were donated by the Hoffman-La Roche Corporation and the Schering Corporation.

by stomach tube, and the effects of pitressin. The responses to ingested water, some after pitressin injections (0.01 U to 1 U per kgm.), were determined for each animal before the use of DCA.

Results. In figure 1 are compared the averaged data of water intake, urine volume, and urine specific gravity for 14 days before the administration of DCA and for a similar period after 6 weeks of daily injections of 2 mgm. of DCA. These 3 dogs were receiving 2 grams of salt per day. The increments in the fluid intake and the urine output, and the fall in the specific gravity of the urine were apparent by the end of the first week.

In dog 2 the DCA injections were discontinued after 74 days. The water intake and urine volume and specific gravity returned to normal within 5 days and there was a loss of 300 grams in body weight. By means of daily injections of DCA, the syndrome of polydipsia and polyuria was maintained in dogs 1 and 3 for more than 160 days. During the last

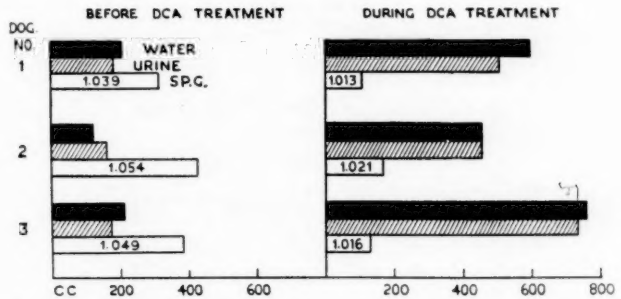

Fig. 1. Average of daily water intake, urine output and urine specific gravity

15 days of a 10 week period of daily injections of 2 mgm. of DCA in dog 1, an increase in the daily salt intake from 2 to 4 grams resulted in a rise in the water intake from 650 to 875 cc. With the salt intake at 4 grams per day, the dose of DCA was increased to 4 mgm. for the next 40 days. There occurred a further increase in the daily water intake from 875 to 2090 cc., a rise in the urine volume from 765 to 1865 cc., while the urine specific gravity fell from 1.010 to 1.005. At this point the added salt (4 grams) was removed from the diet, but injections of 4 mgm. of DCA per day were continued. In 12 days, the water intake fell to 430 cc., and the urine volume to 394 cc. with a rise in its specific gravity to 1.013. Similar results were produced in dog 3, receiving 2 mgm. of DCA, upon the elimination of the 2 grams of sodium chloride from the original diet. *It appears that the magnitude of the excessive fluid exchange which results from small daily doses of DCA is dependent upon the amount of sodium chloride in the diet.*

Two other dogs (4 and 5), on a diet containing 2 grams of sodium chloride,

received injections of 4 mgm. of DCA for 12 days. The data from dog 5 are given in figure 2 and show the manner in which the DI-like syndrome is developed. From either 2 mgm. or 4 mgm. of DCA daily there follows a primary increase in the water intake which is greater than the urinary output. This phenomenon is illustrated in table 1, and demonstrates the primacy of thirst over the polyuria which follows. In our experience, the fall in the specific gravity of the urine has been a sensitive index of DCA action in normal dogs and may prove to be a rapid method for the assay of this type of DCA activity.

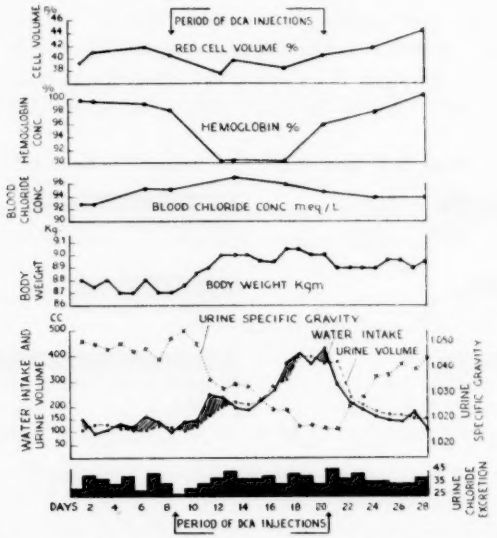

Fig. 2. The evolution and subsidence of polydipsia and polyuria in dog 5 receiving 4 mgm. of DCA and 2 grams of sodium chloride daily. The cross-hatched area between the water intake and urine volume curves indicates a positive water balance.

During the entire injection period, the animals appeared well and showed no "ill" effects, such as muscle weakness or "periodic paralysis" (3). "Heat" occurred in the 3 dogs that received the hormone for the longer periods (74 to 180 days) and was associated with an aggravation of the increase in fluid exchange. After $3\frac{1}{2}$ months of injections and 7 weeks following the last estrus, dog 1 lactated profusely for 3 weeks and then ceased lactating despite the continuance of the hormone. All 5 dogs maintained a steady gain in weight during the administration of the DCA (fig. 2).

When the polydipsia and polyuria were well established, the following special studies were carried out: 1 the effect of complete fluid deprivation

for 1 or 2 days; 2, the effect of water restriction for longer periods; 3, the acute diuretic responses to ingested water; 4, to a 1 per cent saline solution; and 5, the effects of pitressin on water diuresis and on the 24 hour fluid exchange.

1. *The effect of complete fluid deprivation.* After 24 hours without food and water, the DCA injected animals did not elaborate so concentrated a

TABLE 1

Initial water retention and increase in body weight following injections of DCA for 2 to 4 days

DOG NUMBER	DOSE DCA	DAILY FLUID EXCHANGE (WATER INTAKE: URINE OUTPUT) CC.		WATER RETENTION FROM DCA, CC. × DAYS	BODY WEIGHT (KGM.)	
		Average of 7 days before DCA	Average of 2 to 4 days of DCA		Before DCA	After 2 to 4 days, DCA
	mgm.					
3	2	$-45\left(\frac{163}{208}\right)$	$+32\left(\frac{240}{208}\right)$	77 × 3	12.80	12.90
2	2	$-41\left(\frac{127}{168}\right)$	$+7\left(\frac{175}{168}\right)$	48 × 4	10.80	11.25
5	2	$+88\left(\frac{225}{137}\right)$	$+193\left(\frac{345}{152}\right)$	105 × 4	9.40	9.60
5	4	$+3\left(\frac{125}{122}\right)$	$+43\left(\frac{180}{137}\right)$	40 × 3	8.70	8.90
4	4	$+29\left(\frac{196}{167}\right)$	$+80\left(\frac{285}{205}\right)$	51 × 2	8.95	9.15

TABLE 2

Effect of water and food deprivation for twenty-four and for forty-eight hours upon the urine volume and urine specific gravity

DOG NUMBER	BEFORE DCA TREATMENT 24 HOURS		DURING DCA TREATMENT			
	Urine volume	Specific gravity	Urine volume (cc.)		Specific gravity	
			1st day	2nd day	1st day	2nd day
	cc.					
1	80	1.038	180	118	1.021	1.024
2	87	1.043	120	88	1.027	1.029
3	156	1.063	154	136	1.028	1.032

urine as they did prior to the DCA administration. An additional 24 hour deprivation of food and water did not elevate the urine specific gravity to the level obtained in the controls from only 24 hours without food and water (table 2).

2. *The effect of water restriction.* When the daily water intake had risen to about 600 cc. two of the dogs (1 and 3), receiving 2 mgm. of DCA per day, were allowed water in less than half this amount, the diet remaining

unchanged. Figure 3 shows a typical experiment on dog 3 during a 20 day period of progressive water restriction. The results obtained on this dog differ only in detail from those observed on the other animal and on the same dog at a previous experiment. Although dog 3 tolerated water restriction well for 20 days (fig. 3) and a month before for 12 days, dog 1 showed marked thirst and drying of the mouth, nose and vaginal mucous

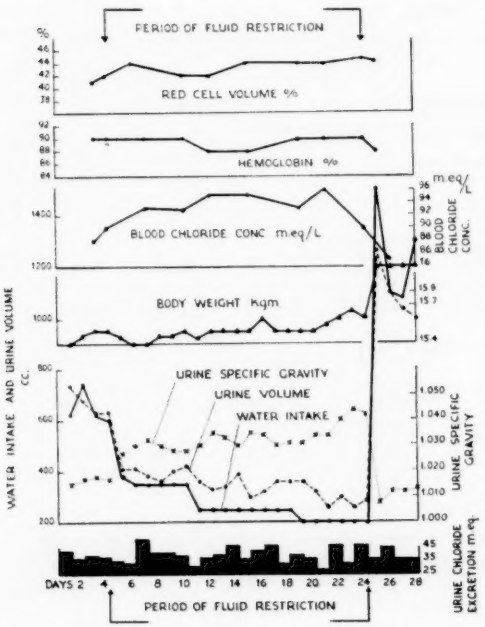
PERIOD OF FLUID RESTRICTION
RED CELL VOLUME %
HEMOGLOBIN %
BLOOD CHLORIDE CONC m.eq/L
BODY WEIGHT Kgm
URINE SPECIFIC GRAVITY
URINE VOLUME
WATER INTAKE
WATER INTAKE AND URINE VOLUME CC.
URINE SPECIFIC GRAVITY
URINE CHLORIDE EXCRETION m.eq.
DAYS 2 4 6 8 10 12 14 16 18 20 22 24 26 28
PERIOD OF FLUID RESTRICTION

Fig. 3

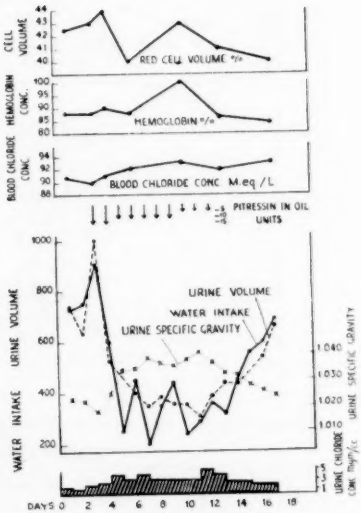
RED CELL VOLUME %
HEMOGLOBIN %
BLOOD CHLORIDE CONC M.eq/L
PITRESSIN IN OIL UNITS
URINE VOLUME
WATER INTAKE
URINE SPECIFIC GRAVITY
DAYS 0 2 4 6 8 10 12 14 16 18

Fig. 4

Fig. 3. Dog 3. Typical response to graduated water restriction in a dog made polydipsic and polyuric by the prolonged administration of 2 mgm. of DCA daily. Compare with figure 4.

Fig. 4. The effects of pitressin in oil on the polydipsia and polyuria induced by the prolonged administration of 2 mgm. of DCA on dog 3. Compare with figure 3.

membranes and inspissation of the stools by the fifth day of water restriction, when the experiment was terminated. The blood chloride concentration had risen from 88 to 101 m.eq. per litre despite the slight and inconstant evidence of hemoconcentration as measured by the hematocrit and hemoglobin values. Upon being allowed water freely this dog drank 1041 cc. of water, voided 440 cc. of urine and gained 700 grams in weight during the following 24 hours period. In neither of the 2 dogs was there any evidence that water restriction altered the urinary excretion of chloride (fig. 3).

3. *Water diuresis.* The responses of 3 of the dogs to a water load of 25 cc. per kilo by stomach tube were determined before and during the administration of DCA (2 mgm.). Qualitatively similar results were obtained in 32 water diuresis tests performed on the 3 dogs before injections were started. Following the administration of the water, there was an increase in the urine volume at the first 30 minute period. The greatest volume of urine was obtained from 1.5 to 2 hours after water administration. By the end of the third hour, the urine volume approached, but usually had not reached the basal level. Despite the constant diet and sufficient water to satisfy thirst, the volume of the control diuresis for the 3 hour period following the administration of the water, was not constant either in the same animal or among the different animals.

The urinary chloride excretion for the 3 hour period of water diuresis varied considerably both in the same animal from day to day as well as among the different animals, although the daily chloride intake remained constant. The amount of chloride excreted during the first half hour after water ingestion equalled and, more frequently, exceeded that of the half hour before water. As the diuresis reached its peak, the chloride excretion diminished in 75 per cent of the tests, but the chloride concentration fell in every experiment. As the diuresis subsided, the chloride concentration rose, but the half hourly excretion remained below pre-diuresis levels.

Twenty-nine water diuresis tests were performed upon these 3 dogs following the development of polydipsia and polyuria from 2 mgm. of DCA and 2 grams of salt. In dog 1 the average diuresis in 3 hours amounted to 91 per cent (56 to 100) of the standard water load (9 expts.) as compared to 80 per cent (42 to 103) before treatment (8 expts.). In dog 2, the average diuresis in 9 experiments was 46 per cent (34 to 65) as compared to 56 per cent (40 to 80) (8 expts.); in dog 3 (11 expts.) it was 78 per cent (56 to 87) as compared to 66 per cent (36 to 91) (17 expts.). The diuresis occurred earlier and with greater intensity during the first hour and subsided more rapidly than in the controls. In only 2 of 31 control tests was there excreted during the first hour more than 40 per cent of the 3 hour urine volume. In the DCA treated animals, 21 out of 29 tests showed a first hour diuresis which was at least 40 per cent of the 3 hour excretion. In the DCA treated dogs the slightly higher levels of urine flow sometimes present in the periods before water ingestion were not sufficiently high to account for the more rapid onset and the greater intensity of the diuresis.

In these dogs the excretion of chloride for the 3 hour diuresis period did not vary appreciably from the controls. The greatest amount of chloride continued to be excreted during the first hour. There was no evidence that the accelerated diuresis which occurred in approximately two-thirds

of the tests on the DCA treated dogs produced a "washing out" of chloride.

The acceleration of the diuretic response occurred during both the moderate and the high increases in the 24 hour water intake and urine output and persisted after the fluid exchange had been markedly reduced by elimination of the extra sodium chloride from the diet. Consequently, the rapidity with which ingested water was excreted by the DCA dogs was not the sole determining factor in the development of the polydipsia. Selye and Basset (1940a, b) observed a similar acceleration of the diuretic response to ingested water both in the intact and the hypophysectomized rat injected with either DCA or progesterone.

4. *Saline diuresis.* One per cent salt solution (25 cc. per kgm.) was administered by stomach tube to 4 normal dogs. Most of the salt solution was retained for more than 3 hours. In 10 experiments the average volume of urine excreted during the 3 hour periods was 19 per cent (11.5 to 29) of the saline administered. In 4 experiments on 2 of the DCA dogs there was excreted 66 per cent (50 to 87) of the saline administered. In the normal dogs, the urine chloride concentration reached a ceiling of about 10 mgm. per cc. and the concentration for the 3 hour period averaged only 8 per cent lower. In the DCA dogs, the maximum chloride concentration varied between 5 and 6.3 mgm. per cc. while the average concentration for the 3 hour period was 4.1 mgm. per cc. This latter was 27 per cent below the maximum and less than half the concentration found in the normal dogs. Despite the lower concentration, the DCA dogs excreted a larger total amount of chloride for the 3 hour period than did the controls. These findings suggest that the depressed urinary chloride concentration which was obtained after the prolonged administration of DCA was due to the inordinate excretion of water.

5. *Effect of pitressin.* Before the administration of DCA the excretion of water administered by stomach tube was inhibited by pitressin (0.1 U to 1 U per kgm. subcutaneously) with a concomitant rise in the specific gravity of the urine, an increase in the concentration and usually in the total excretion of chloride. After prolonged treatment with DCA in dog 1, the injection of pitressin resulted in the same inhibition of diuresis that was observed in the control period before the administration of DCA. However, the urine chloride concentration did not rise so much as in the control tests. In dogs 2 and 3 the pitressin was less effective. In these animals pitressin often caused a marked increase in the excretion of urinary chloride, but the larger volume of urine kept the concentration low. The larger amounts of chloride appearing in the urine may have interfered with the anti-diuretic action of the pitressin, since the latter has little inhibitory effect on salt diuresis (7). However, the fact that pitressin did not increase the urine chloride concentration to the same extent as it did in the controls, indicates that in the 3 DCA dogs injected with pi-

tressin, more water was being excreted than was necessary for the elimination of the chloride.

In 2 of the DCA polyuric dogs, 15 to 45 units of pitressin per day for 3 days resulted in a definite anti-diuretic effect and a rise in the specific gravity of the urine, during the day, when the pitressin was being administered. However, the 24 hour water intake and urine output were not significantly reduced due to an increase in the fluid exchange during the night when no pitressin was given. More prolonged action was secured with pitressin in oil[2] injected in divided doses during the day. In dog 1, 3 cc. were given on the first day, 4 on the second and 2 cc. per day for the next 4 days. By the fourth day there was a gradual reduction in the water intake from 670 to 300 cc., a fall in urine volume from 650 to 252 cc. with a rise in the specific gravity from 1.010 to 1.023 and an elevation in the urine chloride concentration from 2 to 5.3 mgm. per cc. Upon discontinuing injections of the pitressin in oil, 6 days elapsed before the fluid exchange and the urine specific gravity returned to pre-pitressin levels. One month later, a second series of injections of pitressin in oil yielded comparable results. In dog 3, the injection of pitressin in oil in smaller doses produced a decrease in fluid exchange and an elevation in urine specific gravity and urine chloride concentration as depicted in figure 4.

Discussion. Much work has been done on the diuretic response of dogs to a given water load. In the present study, the manner in which the dogs handled a water load of 25 cc. per kgm. intragastrically proved reproducible in each dog from day to day. The facts obtained by such an experiment, when interpreted in the light of the data obtained by 24 hour water studies, together with chloride excretion and specific gravity of the urine, have been so consistent as to make deviation from these data due to the injection of DCA or pitressin of quantitative significance.

The polydipsia and polyuria which developed from the injection of DCA were roughly proportional in degree and in the speed of onset to the daily dose of DCA and to the amount of sodium chloride in the diet. Thus, with the salt intake of 2 grams, 2 mgm. of DCA per day resulted in an increase in the water intake from 152 cc. to 290 cc. in 12 days. On the other hand, 4 mgm. of DCA given for a similar period increased the daily water intake from 125 cc. to 430 cc. Ragan et al. (1940) have shown that during the development of the DI-like syndrome which follows the injection of DCA, there is an elevation of the serum sodium although balance studies showed that there was no great retention of sodium. Our own data show that except for the retention of small amounts of chloride during the first few days of the treatment, the dogs may be kept for several months on 2 mgm. of DCA per day with no significant change in the chloride out-

[2] Supplied through the kindness of Parke, Davis and Company. One cubic centimeter of this product contains 5 to 6 pressor units.

put. The most striking changes that have been obtained by us during the height of the DI-like syndrome from DCA have been a, the consistently low specific gravity of the urine even after marked water deprivation; b, the more rapid excretion of a water load given by mouth; c, the accelerated excretion of saline; and d, the diminished effectiveness of pitressin. These facts, in conjunction with the data obtained during water restriction, are taken to mean that the DI-like syndrome induced by the daily injections of DCA is primarily a disturbance in water metabolism.

Diabetes insipidus produced by operative procedures and the syndrome induced by DCA, resemble each other in many respects. As in DI (8), so in the DCA syndrome, the symptoms are aggravated by increasing the salt intake. Indeed, in one of our experiments in which the water intake had been increased to 2,000 cc. per day by means of 4 mgm. of DCA and 4 grams of salt, removal of the salt from the diet resulted in a virtual "cure" of the DI condition. During fluid restriction, changes in body weight, urine volume and urine specific gravity, comparable to those seen in our dogs, have been observed in dogs with DI by Bellows and Van Wagenen (1938), in DI cats by Fisher, Ingram and Ranson (1938) and in rats with DI by Swann (1939b). Finally, qualitatively similar effects upon water intake, urine volume, urine specific gravity and the concentration of urinary chloride have been produced by pitressin in the polydipsia and polyuria from both DI and DCA.

It has been our working hypothesis that DI is an imbalance between the anti-diuretic activities of the posterior pituitary and the adjacent brain tissue and the diuretic activities of the adrenal cortical hormones. In operative DI the adrenal cortical hormones act without any opposition from the antagonist. In the experiments here recorded, the posterior pituitary is intact since these are normal animals, yet the syndrome of polydipsia and polyuria which has been produced by small doses of DCA has occurred despite the presence of the antagonist. Consequently, quantitative differences between operative DI and the DI-like syndrome produced by DCA should not be construed as against this hypothesis.

SUMMARY

A syndrome of polydipsia and polyuria was produced in 5 normal female dogs by the daily injection of desoxycorticosterone acetate (DCA) in doses of 2 and 4 mgm. The period of hormone injections varied from 12 to 180 days in different dogs and was well tolerated except for the increased fluid exchange.

1. DCA caused an initial increase in the water intake. This was attended by a gain in body weight, a reduction in urine chloride excretion and a fall in the cell volume and hemoglobin concentration of the blood.

2. After a short period of water retention there was an increase in the volume and a decrease in the specific gravity of the urine.

3. The speed of onset and the intensity of the syndrome were roughly proportional to the dose of DCA and the amount of sodium chloride in the diet. The interdependence of sodium chloride and DCA was demonstrated by the amelioration of the severe degree of polydipsia and polyuria after the withdrawal of salt from the diet (2 to 4 grams).

4. When the DCA polydipsia and polyuria were well established, water deprivation failed to elevate the urine specific gravity to normal levels. Water restriction decreased the polyuria but led to thirst and signs of tissue dehydration in the absence of significant hemoconcentration.

5. Water administered by stomach tube was eliminated more rapidly than normally but in no greater amount. The accelerated diuretic response was present whether the syndrome was mild or severe.

6. One per cent saline administered by stomach tube was eliminated more rapidly and in greater quantity than in normal dogs but the maximum chloride concentration in the urine after one per cent saline was lower than in the controls.

7. Pitressin was less effective in inhibiting water diuresis in 2 of 3 polyuric dogs than in the same animals before DCA was administered. After pitressin the urine chloride concentration in the DCA dogs remained below the levels obtained in the controls.

8. Large doses of pitressin in oil reduced the water intake and the urine volume and raised the specific gravity and chloride concentration of the urine.

9. The prolonged use of 2 to 4 mgm. of DCA per day did not result in muscular weakness or other manifestations of toxicity. Upon discontinuance of the DCA injections the water exchange promptly returned to normal.

From the foregoing observations it is concluded that the syndrome of polydipsia and polyuria which had been produced by small doses of DCA in dogs is essentially a disturbance in water metabolism and resembles the diabetes insipidus (DI) syndrome which follows operations upon the hypothalamo-hypophyseal system. We believe that both conditions are due to an imbalance between the physiological activity of the adrenocortical and the posterior pituitary hormones. In operative DI the adrenocortical hormones act unopposed by any secretion from the posterior pituitary, while after DCA administration there is only a relative preponderance of the DCA activity, the end result in each instance being a diabetes insipidus-like syndrome.

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THE TUBULAR REABSORPTION OF UREA, THIOUREA AND DERIVATIVES OF THIOUREA IN THE DOG KIDNEY[1]

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The data reported by Shannon (1, 2) in 1936 and 1938 indicate that the reabsorption of urea in the dog kidney is not an active tubular process but is due entirely to physical diffusion, concurrently with water reabsorption. However, in 1932 Clarke and Smith (3) reported that the elasmobranch tubule reabsorbs almost all of the urea that is filtered at the glomeruli, whereas thiourea is only slightly reabsorbed. If tubular reabsorption of urea in the dog kidney should be in part by means of an active process specific for urea, this might be demonstrated if the reabsorption of a substituted urea were found to be less than that of urea.

There are probably other compounds which are passively reabsorbed in the tubules of the dog kidney. In 1937 Marshall, Emerson and Cutting (4) reported that the sulfanilamide clearance in the dog is increased by increased rate of urine flow, but is independent of the concentration of sulfanilamide in the plasma, which facts indicate that sulfanilamide is passively reabsorbed in the tubules by physical diffusion. But the sulfanilamide clearance was found to be only about fifty per cent of the simultaneous urea clearance. This might be due to a difference between the physical properties of sulfanilamide and urea.

This paper is a report of a study of the tubular reabsorption of urea and certain substituted urea compounds, and of sulfanilamide, in which the factors of rate of water reabsorption and of molecular structure and solubility are given consideration.

EXPERIMENTAL PROCEDURE. Female dogs were used after they had been trained to lie quietly while the renal plasma clearance of thiourea, or of methylthiourea, phenylthiourea, or *s*-diethylthiourea,[2] was determined with the simultaneous urea and creatinine clearances, and sometimes with the simultaneous sulfanilamide clearance, at various rates of decreasing or slowly increasing urine flow. In some experiments these clearances

[1] This study received financial support by grants from the Wisconsin Alumni Research Foundation.

[2] The thiourea, methylthiourea, phenylthiourea and *s*-diethylthiourea were obtained from the Eastman Kodak Company, Rochester, New York.

were determined when the rate of urine flow was forced to increase rapidly. Diuresis was caused by water given orally or by the intravenous infusion of sterile isotonic salt solutions. A rapid acceleration of the rate of urine flow was caused by intravenous infusion of sterile 10 per cent glucose or 18 per cent sucrose solution, 30 cc. per minute for twenty to forty minutes. Urea and also enough creatinine for a plasma creatinine concentration of 10 to 40 mgm. per cent were given orally or intravenously in sterile solution. Thiourea, methylthiourea, or *s*-diethylthiourea were dissolved in sterile water of 60 to 70°C. temperature, and phenylthiourea was dissolved in sterile water of 25 to 35°C. temperature, to avoid decomposition of the solute. For sulfanilamide clearances, one gram was dissolved in about 200 cc. of sterile isotonic saline and the solution injected intravenously. Solutions of thiourea or derivatives of thiourea could not be given orally because they would be regurgitated. Urine was collected by catheter and the bladder was washed twice with 20 cc. of warm, sterile, 1 per cent sodium chloride solution at the end of each collection period. No preservative was added to the portion of each urine to be analyzed for thiourea or the derivative of thiourea which it contained, but toluene was mixed with the remaining portion of each urine to be analyzed for other substances. In the middle of each urine collection period, 12 to 14 cc. of blood were drawn from a femoral artery or vein by syringe, oxalated and centrifuged. The per cent of any constituent of the glomerular filtrate which was reabsorbed in the tubules was calculated by considering the creatinine clearance to be the volume of glomerular fitrate formed in the dog (5).

Creatinine was determined by adaptation of the alkaline picrate colorimetric method (6) to the Evelyn Photoelectric Colorimeter (7) in diluted samples of urine and in the filtrate obtained from 1 cc. of each plasma sample, after the plasma proteins had been precipitated by means of the acid cadmium sulfate method of Miller and Van Slyke (8). Duplicate analyses were made with the following procedure:

> Four cubic centimeters of a solution containing between 1.600 and 0.080 mgm. per cent creatinine are pipetted into a dry photoelectric colorimeter tube. Into another such tube is put 4 cc. of water, to be used as a blank. With each is mixed 2 cc. of alkaline picrate reagent. After fifteen minutes, the galvanometer is made to read 100 with the tube of blank in the colorimeter, using the no. 520 light filter and the no. 6 diaphragm. Then the blank is replaced by the tube of unknown and the galvanometer reading is recorded. From a table of known concentrations of creatinine and the corresponding galvanometer readings the concentration of creatinine in the unknown is found.

Urea was determined in urine by the manometric urease method (9) of Van Slyke. Urea was determined in 2 or 3 cc. of each plasma sample by the aeration-titration urease method (10) of Van Slyke and Cullen.

Sulfanilamide was determined colorimetrically by the method of Bratton and Marshall (11).

A method for the analysis of thiourea and derivatives of thiourea. The methods described in the literature for the quantitative analysis of these thiourea compounds were found unsatisfactory. Therefore a new method was developed which depends upon the color that is produced by the reaction of Grote's reagent (12) with the thiocarbonyl group. This reagent was made as follows the same day it was used:

In a 50 cc. beaker 0.5 gram of sodium nitroferricyanide is dissolved in 10 cc. of water at room temperature. To this is added 0.5 gram of hydroxylamine hydrochloride, which is allowed to dissolve at room temperature. One gram of finely powdered sodium bicarbonate is added to the solution and the beaker is covered with a small watch glass. After evolution of gas has ceased, 0.1 to 0.11 cc. of bromine is added to the solution and the beaker is covered with the watch glass. After evolution of gas has ceased again, the solution is poured into a small flask and gently

TABLE 1

The colorimetric determination of thiourea and derivatives of thiourea

COMPOUND	RANGE	FILTER NUMBER	EQUATION OF RELATIONSHIP (C = MGM. PER CENT OF COMPOUND)
	mgm. per cent		
Thiourea	2–12	580	$C = 44.486 - (22.831 \log G)$
Methylthiourea	2–13	600	$C = 45.763 - (23.320 \log G)$
Phenylthiourea	5–25	620	$C = 89.117 - (45.028 \log G)$
s-Diethylthiourea	2–16	600	$C = 61.783 - (31.694 \log G)$

aerated to remove the excess bromine. Then the solution is filtered into a 25 cc. volumetric flask and diluted to volume. The Grote's reagent should be a clear mahogany brown. It is diluted to one-fifth strength for color development with thiourea or a derivative of thiourea. The one-fifth strength Grote's reagent should not be cloudy, but should have a clear, honey-yellow color. Cloudiness means that insufficient bromine was added, and the reagent will react too slowly and be red in color if too much bromine was added.

After considerable experimentation a reliable method was discovered for the quantitative determination of thiourea or a derivative of thiourea in urine or plasma, which is as follows:

One cubic centimeter of a solution of thiourea or of a derivative of thiourea, within the proper range of concentration (see table 1), is pipetted into the bottom of a dry photoelectric colorimeter tube, and to it is added 1 cc. of one-fifth strength Grote's reagent. The solutions are mixed by gentle rotation of the fluid and allowed to stand sixty minutes. The colored solution is then diluted to 12 cc. with 10 cc. of 1 per cent sodium chloride solution, with immediate mixing by vigorous rotation of the fluid. In fifteen minutes the galvanometer is made to read 100 with a tube of blank in the photoelectric colorimeter, using the proper light filter (see table 1) and the no. 6

diaphragm. If urine or diluted urine is being analyzed, the blank is 12 cc. of water. If blood plasma or plasma diluted with saline is being analyzed, the blank is made from 1 cc. of the plasma or diluted plasma and 11 cc. of 1 per cent sodium chloride solution (hemolysis is avoided as much as possible). Then the blank is replaced by the tube containing the unknown and the galvanometer reading is recorded. The concentration of thiourea or of the derivative of thiourea in the unknown is found by use of a table of known concentrations and the corresponding galvanometer readings, calculated from an experimentally determined equation of relationship (see table 1).

By means of a photoelectric spectrometer[3] the region of maximum light absorption by the colored solution from Grote's reagent and thiourea was identified.

Creatinine in concentrations above 40 mgm. per cent in the 1 cc. of sample was found to interfere somewhat with the reaction between Grote's reagent and thiourea or a derivative of thiourea, causing the galvanometer

TABLE 2

Approximate galvanometer corrections because of creatinine

GALVANOMETER READING OBSERVED	CREATININE IN SAMPLE (MGM. PER CENT)				
	50	100	150	200	250
	Amount to be subtracted to correct the reading				
25.0	−1.0	−2.25	−3.5	−4.75	−6.0
35.0	−1.5	−2.75	−4.0	−5.25	−6.5
45.0	−2.0	−3.25	−4.5	−5.75	−7.0
55.0	−2.5	−3.75	−5.0	−6.25	−7.5
65.0	−2.75	−4.0	−5.25	−6.5	−7.75
75.0	−3.0	−4.25	−5.5	−6.75	−8.0

reading to be a little too high. Therefore the corrections given in table 2 were determined by experiment and interpolation.

The solubilities of the thiocarbonyl compounds and of sulfanilamide in water and in ether saturated with water were determined by allowing warm saturated solutions of these substances, with undissolved material present, to cool to the room temperature of 24°C. The saturated solutions were then analyzed. The results are in table 3.

Experiments were performed to determine whether or not the thiocarbonyl compounds are metabolized by the dog after they are injected intravenously. In each experiment a known quantity of the thiocarbonyl compound in sterile solution was injected intravenously. After about one hour the plasma concentration of the compound and the quantity which had been excreted in the urine were determined. The calculated

[3] Through the courtesy of Dr. V. W. Meloche of the Department of Chemistry, University of Wisconsin, use of this apparatus was obtained.

remainder divided by the plasma concentration gave a figure which might have been the volume of body water in which the compound was evenly distributed. Collection of the urine was continued for two or three hours longer and the plasma concentration and quantity of the compound which had been excreted in the urine were determined. The calculated remainder divided by the plasma concentration gave nearly the same figure as had been obtained from the previous data, which indicated that the compound had not been metabolized during the experiment. The thiocarbonyl compounds seem not to be metabolized in the dog during three or four hours after intravenous injection of them.

RESULTS. Some of these thiocarbonyl compounds produced certain pharmacological effects in the dogs. The intravenous infusion of an isotonic solution containing phenylthiourea caused repeated vomiting and

TABLE 3

Properties and renal excretion of urea, thiourea and derivatives of thiourea, and sulfanilamide

SOLUTE	TUBULAR REABSORPTION OF FILTERED SOLUTE	CLEARANCE RATIO $\frac{\text{SOLUTE}}{\text{UREA}}$	SOLUBILITIES (MILLIMOLS PER LITER)		RATIO OF SOLUBILITIES WATER/ETHER
			In water (24°C.)	In ether (24°C.)	
	per cent				
Urea	18–60		18,833	2.35	8000/1
Thiourea	20–59	Mean = 1.007	2,217	19	116/1
Methylthiourea	29–87	0.88–0.39	1,982	40	49/1
Sulfanilamide	34–61	0.88–0.50	61.6	12.3	5/1
Phenylthiourea	28–93	0.79–0.15	21.7	31	1/1.4
s-Diethylthiourea	61–99	0.48–0.02	326	847	1/2.6

retching in a dog, followed by its death within twenty-four hours. In later experiments the infusion was discontinued as soon as the dog vomited once. Thereafter, only one more death occurred from phenylthiourea poisoning and no deaths were caused by the intravenous injection of any of the other compounds used. Methylthiourea caused vomiting when the plasma concentration exceeded 70 mgm. per cent. *S*-diethylthiourea caused vomiting when the plasma concentration was greater than 15 mgm. per cent. Phenylthiourea caused vomiting when the plasma concentration was 4 mgm. per cent or more.

The clearances and the tubular reabsorption of urea, thiourea and the derivatives of thiourea were found to be independent of the plasma concentrations of the respective solutes within the ranges obtained, which were as follows: For urea, 40 to 410 mgm. per cent; for thiourea 15 to 145 mgm. per cent; for methylthiourea, 15 to 110 mgm. per cent; for phenyl-

thiourea, 4 to 10 mgm. per cent; for *s*-diethylthiourea, 12 to 26 mgm. per cent.

The per cent of water reabsorbed in the tubules was found to have a pronounced effect upon the percentages of thiourea and the derivatives of thiourea reabsorbed. The finding of Shannon (1) that the urea clearance is affected by a change in the rate of urine flow and the finding of Marshall, Emerson and Cutting (4) that the sulfanilamide clearance is similarly affected, were verified. When the per cent of water reabsorbed was low, and the urine flow consequently high, the percentages of these solutes reabsorbed were low and their clearances, consequently, high. On the other hand, when the per cent of water reabsorbed was high, the percentages of these solutes reabsorbed were high and their clearances, consequently,

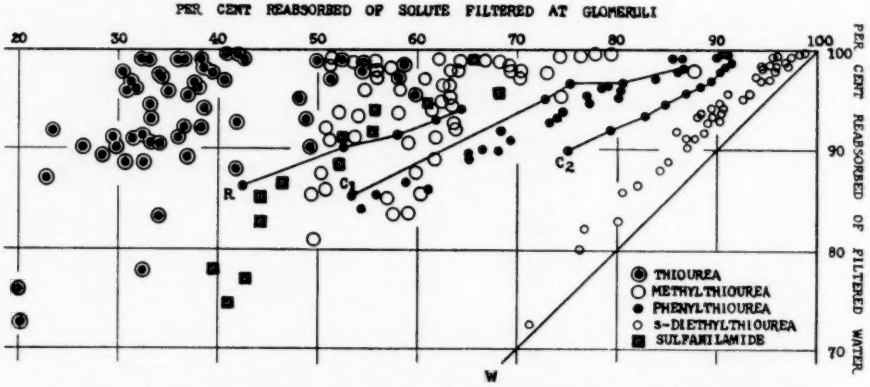

Fig. 1. The tubular reabsorption of thiourea and derivatives of thiourea when the rate of urine flow is decreasing or only slightly increasing.

low. In figure 1 the percentages of the thiocarbonyl compounds and of sulfanilamide reabsorbed in the tubules are plotted against the percentages of water reabsorbed, when the rate of urine flow is decreasing or only slowly increasing. The diagonal line *W* is the line upon which all points would fall, theoretically, for a substance in the glomerular filtrate, the percentage reabsorbed of which would always equal the percentage reabsorbed of the tubular water. *S*-diethylthiourea is reabsorbed very nearly like such a theoretical substance. There is a striking rectilinear increase in the percentages reabsorbed of both *s*-diethylthiourea and phenylthiourea as the per cent of water reabsorbed increases. Small solid black circles connected by a line represent observations from a single experiment with phenylthiourea upon the dog whose initial is printed nearby. Two different experiments upon the same dog C are marked, respectively, by C_1 and C_2.

The percentages of thiourea and urea reabsorbed were found to be very

nearly the same during the same interval of time. The arithmetical mean of fifty-four ratios of the thiourea clearance to the urea clearance in periods with decreasing or slowly increasing rates of urine flow was found to be 1.007, or nearly unity. The distribution of these clearance ratios about the arithmetical mean was found to be that distribution which would be expected when the deviations from the mean are due to experimental error. Two-thirds of the clearance ratios were between the limits of 0.926 and 1.088.

The per cent of each derivative of thiourea reabsorbed was always greater than the per cent of urea reabsorbed during the same period, and, consequently, the clearance of each derivative of thiourea was always less than the simultaneous urea clearance, as shown by the clearance ratios in table 3. The simultaneous sulfanilamide and methylthiourea clearances were found to be very nearly equal. At usual low post-absorptive rates of urine flow the methylthiourea clearance was found to be about 50 per cent, the phenylthiourea clearance about 20 per cent, and the *s*-diethylthiourea clearance about 3 per cent of the simultaneous urea clearance. The ranges of the ratios of these clearances to the simultaneous urea clearance during decreasing or slowly increasing rate of urine flow are given in table 3.

Shannon (1) studied the striking exaltation of the urea clearance which is evoked by an acceleration of the rate of urine flow and reported that the urea clearance often began to decrease from the maximum value attained, even though the rate of urine flow continued to increase for a short time. In our investigations, this phenomenon was found to be most pronounced during a massive infusion of 10 per cent glucose or 18 per cent sucrose solution. The phenomenon was found to occur later with thiourea than with urea, i.e., the ratio of the simultaneous urea and creatinine clearances was observed to reach a maximum value and then to fall, before the ratio of the simultaneous thiourea and creatinine clearances reached a maximum value. This exaltation phenomenon has not been observed at all with the derivatives of thiourea or with sulfanilamide. With these latter compounds the curve obtained while the rate of urine flow increased was found to coincide, approximately, with the curve obtained while the rate of urine flow decreased, although in every one of these experiments a pronounced exaltation of the urea clearance was observed while the urine flow rapidly increased. In figure 2 the data from a representative experiment with urea and thiourea are plotted and also the data for each of the three derivatives of thiourea from respective representative experiments.

Discussion. The belief that there is no active process of tubular reabsorption of urea is supported by the fact that during decreasing or slowly increasing urine flow the reabsorption of thiourea is approximately the same as that of urea, and furthermore, by the fact that the per cent

of each derivative of thiourea reabsorbed is greater than the per cent of urea reabsorbed during the same period. One might expect that if urea were reabsorbed in part by an active tubular process, characteristically specific for urea, the greater the modification of its chemical structure the less would be its reabsorption.

Thiourea, methylthiourea, phenylthiourea and *s*-diethylthiourea seem to be reabsorbed in the tubules by physical diffusion and not by active tubular reabsorption. Hence the mechanism of renal excretion of thiourea and derivatives of thiourea seems to be similar to the mechanism of renal

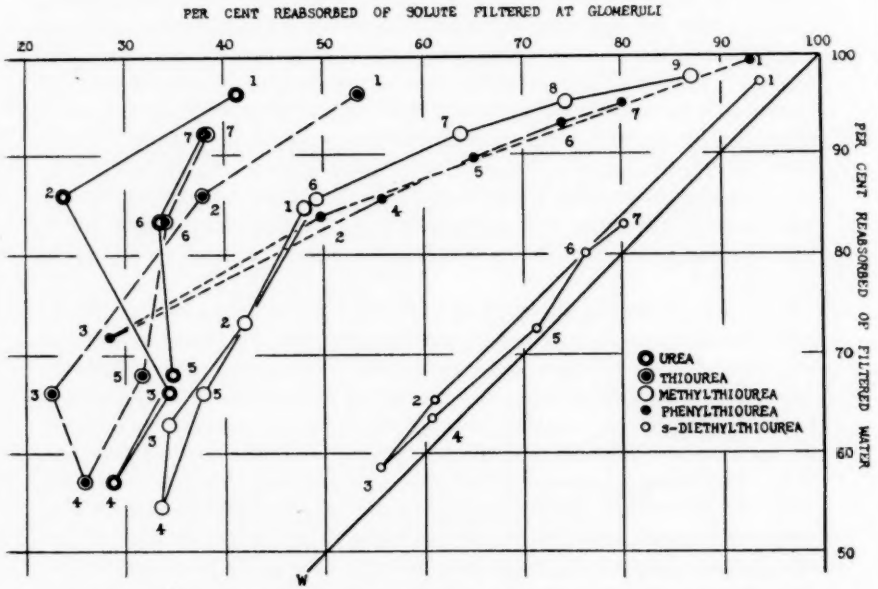

Fig. 2. The effect of rapid acceleration of the rate of urine flow upon the renal excretion of urea, thiourea, and derivatives of thiourea.

excretion of urea (1, 2) and of sulfanilamide (4). These compounds seem to be filtered at the glomerulus and passively reabsorbed in part, concurrently with the reabsorption of water.

A direct relationship between molecular structure and the per cent reabsorbed of urea, thiourea and the derivatives of thiourea, and of sulfanilamide was found in these experiments. Thiourea and urea, of which the same per cent is reabsorbed during decreasing or slowly increasing rate of urine flow, have two unsubstituted amino groups per molecule. The fact that the per cent of phenylthiourea reabsorbed at low urine flows is greater than the per cent of methylthiourea reabsorbed seems to

be due to the fact that the phenyl radicle is larger than the methyl radicle. Like methylthiourea and phenylthiourea, sulfanilamide has only one unsubstituted amino group per molecule (although sulfanilamide has an unsubstituted amide group as well, with, it seems, properties quite different from those of the amino group), which seems to make possible a percentage of reabsorption which is greater than that of urea. It is striking that *s*-diethylthiourea, which has neither of its amino groups unsubstituted, has the greatest percentage of reabsorption.

It seems that the tubular epithelium has a boundary which resists the diffusion of molecules with free amino groups through it. The nature of this boundary seems to be lipoid, because a comparison of the solubilities of these compounds in water, and in ether saturated with water, with their molecular structures indicates that the unsubstituted amino groups contribute to the relative water-soluble, ether-insoluble nature of the compounds. This gives support to the belief that the more water-soluble and lipoid-insoluble a constituent of the glomerular filtrate is, the lower will be the percentage of its passive tubular reabsorption. This may explain why Pitts (13) found the hexamethenamine clearance in the dog to be, like the clearance of urea, unaffected by the drug phlorizin and independent of the plasma concentration of hexamethenamine, but greater than the urea clearance and less than the glomerular filtration. Apparently, hexamethenamine has a very low, but definite, percentage of reabsorption at ordinary low urine flows in the dog, due, perhaps, to the fact that the compound is only very slightly lipoid (ether) soluble, but quite soluble in water (14).

The exaltation of the urea and thiourea clearances, which occurs when the rate of urine flow is accelerated, is a phenomenon which seems to be related to the relative water-solubilities of these compounds, because it occurs earlier with the most water-soluble of the two compounds and not at all with the derivatives of thiourea, which are less water-soluble than thiourea. This transitory exaltation of the clearance is tentatively explained as follows: The sudden decrease in water reabsorption that follows forced diuresis may almost stop the diffusion of the molecules of urea in the tubule wall next to the lumen. The increase in flow of the tubular fluid may then literally wash these molecules out of the wall because they are so soluble in water, thereby adding them to the molecules which have been more recently filtered at the glomerulus, thus effecting a marked increase in the clearance. The clearance falls from the maximum elevation, even though the urine flow still increases, when the tubule wall has been washed quite free of urea, so that the urea filtered at the glomerulus is no longer augmented by that washed from the tubule wall. The transient exaltation of the thiourea clearance during forced diuresis may be similarly explained, except that the tubule wall seems to be washed free

of thiourea later than it is washed free of urea, because thiourea is not as water-soluble as is urea; therefore, the maximum thiourea clearance occurs later than the maximum urea clearance.

SUMMARY

The renal plasma clearance of each of the following compounds, thiourea, methylthiourea, phenylthiourea or *s*-diethylthiourea, was determined with the simultaneous urea and creatinine clearances. The per cent of water which was reabsorbed in the tubules was found to have a pronounced effect upon the percentages of urea, thiourea and the derivatives of thiourea which were reabsorbed. From low to high percentages of water reabsorbed, 61 to 99 per cent of the *s*-diethylthiourea, 28 to 93 per cent of the phenylthiourea, 29 to 87 per cent of the methylthiourea, 20 to 59 per cent of the thiourea, and 18 to 60 per cent of the urea filtered at the glomeruli were reabsorbed. The striking exaltation of the clearance, evoked by an acceleration of the rate of urine flow, was found to occur later with thiourea than with urea, but has not been observed at all with the derivatives of thiourea.

The percentages of thiourea and urea reabsorbed are approximately the same during decreasing or slowly increasing rate of urine flow. The per cent of each derivative of thiourea reabsorbed is always less than the per cent of urea reabsorbed during the same interval of time, and at normal, low rates of urine flow the methylthiourea clearance is about 50 per cent, the phenylthiourea clearance is about 20 per cent, and the *s*-diethylthiourea clearance is about 3 per cent of the simultaneous urea clearance. The reabsorption of thiourea and of each of its derivatives was found to be independent of the plasma concentration obtained of each substance, respectively, in these experiments.

The relative magnitudes of the percentages of thiourea and the derivatives of thiourea reabsorbed directly correspond to the chemical structures of the molecules, with reference to the substituted and unsubstituted amino groups, and seem to be related to the ratios of water-solubility to ether-solubility of the compounds.

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THE EFFECT OF NEPHRECTOMY ON THE BLOOD PRESSURE RESPONSE TO RENIN AND ANGIOTONIN[1]

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Since persistent arterial hypertension has been studied intensively, a large number of substances have been suggested as possible chemical mediators for this disorder. However, none of these has been generally accepted as an etiologic factor in clinical hypertension and no crucial evidence has as yet been advanced to indicate the relationship between the increased arterial pressure and the various suggested agents.

Although a protein-like substance extracted from kidneys, renin, was suggested (1) as a mediator responsible for the regulation of the blood pressure long ago, the characteristics of this agent have only recently been vigorously reinvestigated. Improvements in the methods of extraction (2, 3, 4) with removal of depressor materials which were responsible for much of the controversy in the earlier literature have led to a fuller understanding of the nature of this substance.

The close relationship between the kidney and hypertension, reëmphasized by the recent development of a satisfactory method for the production of persistent arterial hypertension by means of partial occlusion of the renal artery (5) has further increased the interest in renin. This method has also been useful in affording an opportunity to study by indirect means some of the properties of the chemical mediator of renal hypertension.

Since we have found that the chemical mediator of hypertension is destroyed at a rapid rate only by the renal metabolic processes, it would be expected that a substance proposed as a likely etiologic agent in hypertension should have an exalted pressor response in the totally nephrectomized animal. We have undertaken to determine whether or not this potentiation occurs in the dog injected with renin.

Blood pressures were determined with the Hamilton manometer (6) on the trained unanesthetized dog, according to the method previously described (7). Only when the diastolic blood pressure varied less than plus or minus 5 mm. Hg on several successive readings was the animal subjected

[1] Aided by the A. D. Nast Fund for Cardiac Research and a grant from Eli Lilly and Company.

to the experimental regime. With the dog lying quietly on its side, the needle of the manometer was inserted into the femoral artery, and the cyclic variations recorded optically on photosensitized paper. A small amount of heparin (Liquemin[2]) was added to the citrate to help prevent clotting and allow a prolonged record to be taken. After two minutes of control readings, the sample of renin was injected over ten to fifteen seconds into the basilic vein. Blood pressures were recorded at intervals of 15 to 60 minutes until the pressor effect was dissipated. Renin was prepared according to the methods of Helmer and Page (3)[3] and Landis, Jeffers and Shiels (4).

Total nephrectomies were performed using ether anesthesia in order to ensure adequate elimination of the anesthetic agent. Atropine was used in some instances to prevent salivation which occurs with ether anesthesia. Aseptic technique was used throughout. Injections of renin or angiotonin were made at intervals of 24 hours until the animal succumbed in uremia or was sacrificed.

The intensity of response was measured in terms of the difference between the maximal systolic and diastolic pressures and the preinjection control levels. The duration of the response was measured in terms of the time necessary for the diastolic pressure to return to the control level.

Renin. The effect of renin was tested in 13 experiments. Although the same dog received aliquot portions of the same renin solution each day, equivalent doses were not used on different animals. The doses ranged from 0.04 cc. to 1 cc. The maximal response was attained in 2 to 3 minutes after the injection and the effect was usually dissipated in 1 to 3 hours.

a. *Effect of daily injections of renin into the intact animal.* Renin was injected at 24 hour intervals into 3 dogs for 2, 3 and 3 days respectively. In dog 1 the maximal responses were identical on both days. The blood pressures began to fall at similar rates on both days, but after the second injection the pressure had remained above the control level. In dog 2 no change in the intensity of response was seen, on the three successive days, but the duration increased somewhat with each successive injection. In dog 3 the response was identical in intensity and duration on the three successive days.

b. *The effect of a single injection of renin before and after nephrectomy.* In five dogs (4, 5, 6, 7 and 8) intensity and duration of response to renin injection was identical before and after nephrectomy. The maximal blood pressure increase in these animals was 45/45 mm. Hg in three dogs, 70/70 in one and 60/90 in one. All these animals received injections on

[2] We are grateful to Roche-Organon, Inc. for supplies of Liquaemin.

[3] We wish to thank Dr. I. H. Page for the generous supplies of renin and angiotonin used in this study.

the day preceding and following the nephrectomy. One was tested also on the second day and one on the second and fourth days.

In two other dogs with distemper (9 and 10) renin injection 24 hours after nephrectomy gave an increased response in both intensity and duration. However, in these animals the basal blood pressure on the first day following nephrectomy had fallen close to shock level. The renin injection which was adequate to provoke only a small response before the operation now raised the blood pressure to the original normotensive levels. The rate of drop of the blood pressure was approximately the same as in other animals with post-operative normal blood pressure levels.

The injection of renin into one other dog (11) with distemper gave rise to similar blood pressure changes before and after nephrectomy. 90 minutes after the post-nephrectomy injection this animal went into shock, the blood pressure fell to 95/50 mm. Hg and was maintained at this level until the animal died several hours later.

Two dogs (1, 2) previously used for control injections were again injected with renin for three days just prior to nephrectomy. After the operation, similar quantities were injected on the first and second post-operative days in both and also in the fourth day in one. The response is illustrated in table 1.

In dog 1 the intensity of response on renin injection showed a considerable progressive increase, viz., 30/45, 75/70 and 110/95 mm. Hg, above the control level on the three pre-operative days. The duration of the response also increased from 150 to 245 to 270 plus minutes on the successive days. After nephrectomy the intensity of response was of the order of the two days previous to the nephrectomy, while the duration was increased further beyond that pre-operatively (table 1).

Our results indicate that total nephrectomy did not significantly increase the response to renin in most of our dogs. A mildly increased response to renin injection may occur in some dogs if the injections are made daily, whether the kidneys are present or not; in other dogs no such increase in response is seen.

In the first report on renin (1) it was stated that nephrectomized rabbits gave a much greater response in intensity and duration than did normal rabbits. Merrill, Williams and Harrison (9) found that clamping the renal pedicle of rats did not immediately affect the response, but after two or three days an increased response was seen, and Freedman (10) confirmed these findings in the unanesthetized rat. This increased response was believed to be due to the exhaustion of a depressor material normally manufactured by the kidney. In dogs, Hessel (2) found no difference in response after nephrectomy, Wakerlin and Chobot (11) found a potentiated response one to three hours after nephrectomy and Page and Helmer (8) found no significant change in the early hours after nephrectomy while an increase in response was seen after 24 to 48 hours.

TABLE 1

Effect of nephrectomy on the blood pressure response to intravenously injected renin in typical experiments

DOG NO.	DAY	PRENEPHRECTOMY							POSTNEPHRECTOMY						
		Control blood pressure, mm. Hg		Peak blood pressure, mm. Hg		Levels of blood pressure at various times after renin injection, mm. Hg			Control blood pressure, mm. Hg		Peak blood pressure, mm. Hg		Levels of blood pressure at various times after renin injection, mm. Hg		
		Sys.	Dias.	Sys.	Dias.	Min.	Sys.	Dias.	Sys.	Dias.	Sys.	Dias.	Min.	Sys.	Dias.
7	1	150	80	210	150	30	200	135	200	110	275	175	55	200	130
						95	150	80					95	175	110
													135	165	110
6	1	125	65	175	120	90	135	80	115	80	160	130	45	160	135
						120	115	50					90	95	50
						150	130	70					165	100	55
5	1	200	90		190	35	200	110	200	115	250	200	60	200	125
						95	200	105					100	180	100
													150	190	115
	2								200	110	250	200	7	240	160
													50	200	125
													90	185	130
													150	200	120
2	1	135	85	250	160	30	150	120	175	85	250	175	50	200	125
						60	140	100					110	185	110
						90	120	80					180	195	125
						150	135	95					240	185	125
	2	150	85	250	165	90	180	115	180	95			120	200	130
						135	155	100					240	200	120
						240	150	100					360	195	120
	3	150	85	250	165	60	195	95							
						120	185	100							
						180	175	110							
1	1	160	85	190	130	34	200	120	155	75	225	155	70	210	120
						54	180	105					130	200	120
						94	170	95					200	190	105
						150	150	85					270	170	100
	2	175	90	250	170	80	205	120	160	85			130	200	125
						170	175	105					250	185	125
						245	175	100					385	190	120
	3	140	75	250	165	85	185	105	190	105		175	125		135
						140	165	95					275		125
						200	185	95					470		125

Control blood pressure is the pressure just previous to the injection.
Peak blood pressure is the maximum pressure after injection.
Sys. is systolic and dias., the diastolic pressure.

This discordance in results and interpretation suggests that other factors may be operative. The differences may be due to the different species used and the magnitude of change which each worker considers significant. Some of the discrepancies may be due to the daily variability in the response of the animals, the effect of anesthesia and the summation of the renin effect with the neurogenic rise in blood pressure which may follow in the first few hours after nephrectomy (7).

Angiotonin. The first suggestion that another factor dependent upon the kidney other than renin might play a rôle in hypertension was presented by Petrowsky (13) who found a thermostable, Berkefeld filterable pressor substance in the Ringer-Locke perfusate of kidneys. The presence of a non-protein pressor substance in kidney autolysates which was more heat resistant than renin was reported by Hartwich and Hessel (14) with the suggestion that this might be the pressor principle of renin. Williams and Grossman (15) found an adrenalin-like substance in the Ringer's perfusate of kidneys, especially if these kidneys were allowed to autolyze for two hours or more before beginning the perfusion. Using the isolated dog tail, Friedman, Abramson and Marx (16) reported that renin has no activity on perfusion with Ringer's solution while it is a potent vasoconstrictor if perfused with blood.

The significance of these findings became clearer with the almost simultaneous announcements from two laboratories (17, 18) that the interaction of renin with an activating substance in the blood gave rise to a new heat stable pressor material. This substance has been called Angiotonin or Hypertensin.

The possibility that this substance might fulfill the criteria of the chemical mediator of nephrogenic hypertension by giving a potentiated response in nephrectomized dogs caused us to repeat our experiments with angiotonin.

Angiotonin was injected on subsequent days into three dogs for 2, 3 and 3 days respectively, without variation in the blood pressure response. The maximal rise in pressure was usually attained in 30 to 45 seconds after injection and the blood pressure returned to basal levels in 3 to 8 minutes.

Nine dogs (nos. 14 to 23) received a single control injection shortly before nephrectomy. After operation these animals received daily injections of angiotonin for 2, 2, 2, 3, 3, 3, 4, 5 and 5 days respectively. The response was essentially the same in intensity and duration with that seen on the control day.

Three animals received more than one daily injection before the nephrectomy. The results on these dogs are summarized below:

In dog 24, no change in response was noted between the nine pre-operative and three post-operative injections (table 2). In dog 25, previously uninephrectomized, the duration of the response prior to and after nephrec-

tomy was identical except on the fourth post-operative day when a rise of diastolic pressure of 25 mm. Hg was still evident at the eighth minute. After injection of angiotonin on the fifth post-operative day, as the blood pressure reached its peak, cardiac slowing leading to asystole set in with a rapid fall in blood pressure to zero. After about ten seconds the dog became restless, dyspneic, went into opisthotonic rigidity and expired.

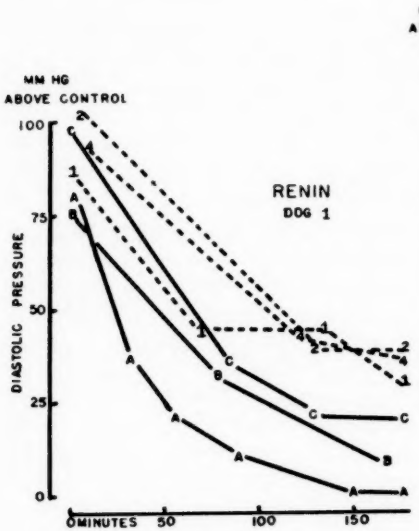

Fig. 1

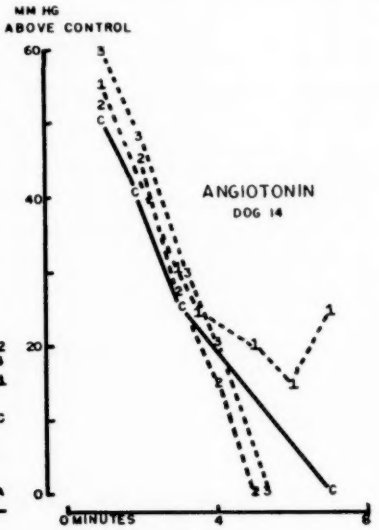

Fig. 2

Fig. 1. The effect of injection of renin into a dog on successive days. Ordinates give diastolic blood pressure above control level, abscissa, time in minutes. The lines indicated by the marks *A–A–A*, *B–B–B*, *C–C–C* represent the level of the diastolic blood pressure above the control level on the 3rd day, 2nd day and the day before nephrectomy, respectively. *1–1–1*, *2–2–2* and *4–4–4* represent the same values on the first, second and fourth days after nephrectomy, respectively. Discussed in text.

Fig. 2. The effect of injection of angiotonin into a dog on successive days. *C–C–C*, *1–1–1*, *2–2–2* have the same significance as in figure 1. *3–3–3* represents the third day after nephrectomy. Discussed in text.

Dog 26 received a dose of angiotonin for two days and the pressure changes were identical. Right nephrectomy was then performed and the same amounts of angiotonin were injected daily for seven more days giving rise to essentially the same response. The remaining kidney was then removed. Daily injections for 2 days again gave the same response.

In our hands, then, total nephrectomy did not significantly affect the intensity or duration of the blood pressure response to angiotonin injections. There was no increased response as uremia developed. Such an

increase would be expected if the blood concentration of a postulated inhibitor of renin or angiotonin were decreased, as a result of removal of

TABLE 2

Effect of nephrectomy on the blood pressure response to intravenously injected angiotonin showing a typical response

DOG NO.	DAY	PRENEPHRECTOMY							POSTNEPHRECTOMY						
		Control blood pressure, mm. Hg		Peak blood pressure, mm. Hg		Levels of blood pressure at various times after angiotonin injection, mm. Hg			Control blood pressure, mm. Hg		Peak blood pressure, mm. Hg		Levels of blood pressure at various times after angiotonin injection, mm. Hg		
		Sys.	Dias.	Sys.	Dias.	Min.	Sys.	Dias.	Sys.	Dias.	Sys.	Dias.	Min.	Sys.	Dias.
24	1	145	75	160	110	2	140	85	130	75	155	95	2	150	80
						3	130	80					3	130	80
						4	135	75					4	135	75
	2	145	75	200	125	2	185	110	110	70	125	85	2	125	75
						4	155	100					3	125	75
						5	195	95					4	110	70
													5	110	70
	3	140	75	175	110	2	150	90	85	65	115	80	2	105	75
						3	145	80					3	100	75
						4	140	80					4	95	70
													5	90	60
													6	85	60
	5	175	105	200	135	2	175	115							
						3	170	110							
	6	150	80	175	110	3	145	80							
	7	175	90	175	120	2	150	100							
						4	175	100							
						5	145	75							
	8	145	80	150	100	2	150	95							
						3	145	95							
						5	145	75							
	9	150	90	165	115	2	150	95							
						3	145	90							
						4	135	85							

Control blood pressure is the pressure just previous to the injection.
Peak blood pressure is the maximal pressure after injection.
Sys. is systolic and dias., the diastolic pressure.

their source, the kidneys. The changing basal level of the blood pressure sometimes seen from day to day in unanesthetized dogs had no effect on the rise in blood pressure attendant upon angiotonin injection.

It has been reported (17, 18) that an increased response to angiotonin occurs after nephrectomy and this potentiation has been believed to be due to the exhaustion of an anti-angiotonin substance which is produced by the kidneys. Inspection of the published data indicates that their significance may be equivocal and the changes may be a result of biological variation.

SUMMARY

The intensity and duration of the blood pressure response to renin is essentially the same before and after nephrectomy. In some animals, however, the duration of the response is somewhat increased following nephrectomy.

Renin has many properties which would suggest that it is closely related to the mechanism responsible for arterial hypertension of renal origin. Among these are the consistent presence of renin in kidney extracts, the reaction of renin with some of the constituents of the blood (renin-activator) to produce a new pressor material (angiotonin), and the reduction in intensity of response to renin seen in adrenalectomized animals. Nevertheless it appears that renin has not fulfilled one of the criteria necessary for the substance responsible for hypertension, since no consistent potentiation of the intensity or duration of the blood pressure response occurs after nephrectomy. The increase in duration of the response seen in some animals after nephrectomy is not of the magnitude predicted for the mediator of hypertension by our previous experiments (12).

The similarity in the blood pressure response to angiotonin before and after total nephrectomy suggests that the kidney is not responsible for the destruction or elimination of this substance.

I wish to express my gratitude to Dr. L. N. Katz for his advice and guidance and my indebtedness to Miss L. Friedberg and Messrs. R. Asher and H. Philipsborn for their assistance.

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LIPOCAIC AND KETONEMIA IN PANCREATIC DIABETES[1]

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Evidence for the existence and concerning some of the properties of lipocaic as an internal secretion of the pancreas distinct from insulin has been presented in previous publications from this laboratory (1). While knowledge concerning the function of this substance is still incomplete there is considerable evidence indicating that it exerts a definite and specific influence on fat metabolism. A profound hypolipemia and fatty infiltration in the liver develop in depancreatized dogs with lipocaic deficiency but who receive adequate insulin therapy. The administration of lipocaic to such animals elevates the blood lipids to normal and clears the liver of fat. At the same time there is evidence that a considerable amount of sugar is made available for metabolism and it seems probable that this is derived from the large store of fat that leaves the liver. During the period of lipocaic deficiency when fat is accumulating in the liver a progressive decrease in insulin requirement and tolerance develops and also a progressive decrease in glucose excretion. This cannot be accounted for by decreased intake of food and is more probably due to an interference with the overproduction of glucose by the liver as a result of the fat deposit. When lipocaic is given the excretion of glucose rises sharply from a level of 3 to 5 grams to 30 grams or over per 24 hour period and the insulin tolerance from less than 5 units daily to 30 or 40. Both of these events testify to the appearance of large amounts of glucose in the metabolic process without a corresponding increase in intake. The large amount of glucose would seem to demand the conversion of fatty acid as well as glycerol under the influence of lipocaic.

It seems to be established that the liver is the chief source of the ketone bodies and that the fatty liver forms more ketone bodies than one rich in glycogen. Soskin's excellent critical review (2) indicates that the former concept of the ketone bodies as abnormal products of incomplete fat oxidation which accumulate in diabetes because of inadequate carbohy-

[1] This work has been aided by grants from the Committee on Research in Endocrinology of the National Research Council, the Douglas Smith Foundation for Medical Research of The University of Chicago and the Eli Lilly Company.

drate oxidation must yield to the view which regards these substances as normal intermediate metabolites which appear during the conversion of fatty acid to sugar. Accordingly it appeared to us of considerable importance to determine the relation, if any, between lipocaic and ketogenesis. The concentration of ketone bodies in the blood of depancreatized dogs was determined under the following conditions: *a*, depancreatized dogs adequately controlled with both insulin and lipocaic; *b*, depancreatized dogs receiving adequate insulin but no lipocaic; *c*, depancreatized dogs receiving adequate lipocaic but inadequate insulin; *d*, depancreatized dogs receiving no lipocaic and inadequate insulin, and *e*, depancreatized dogs with fatty livers of lipocaic deficiency adequately controlled with insulin and given curative doses of lipocaic.

EXPERIMENTAL PROCEDURE. Data secured from 52 completely depancreatized dogs form the basis for this report. These animals were fed a

TABLE 1

Summary of data showing that the changes in blood ketones are due to insulin and not lipocaic

TREATMENT RECEIVED	INSULIN	BLOOD KETONES	URINE SUGAR DAILY
	units	*mgm. per 100 cc.*	*grams*
12 dogs: adequate insulin; adequate lipocaic..	30	5.7	8.0
5 dogs: adequate insulin; no lipocaic.....	22	3.5	6.0
13 dogs: inadequate insulin; adequate lipocaic.....	9	21.0	25.0
20 dogs: inadequate insulin; no lipocaic.....	8	14.7	23.0
2 dogs with fatty livers. Then given adequate insulin and adequate lipocaic.....	24	5.7	7.0

diet of bread, milk, ground beef and beef fat, with cod liver oil and brewer's yeast as vitamin supplements. In addition many of them were given fresh active dog pancreatic juice in varying amounts (50–200 cc. daily) to improve digestion and absorption. This diet has been found to contain from 25 to 40 per cent fat. Daily determinations were made of glucose excretion for each animal with occasional estimations of the blood sugar. Regular insulin was used and injections were given twice daily as a rule. Ketone bodies were determined by the method of Barnes and Wick (10) on blood samples secured usually once a week from each animal. The data are summarized in table 1 in which averages are given for the amounts of insulin, the level of the blood ketones, and the glucose excretion, for the periods of study under each condition. These periods lasted as a rule from two to three months except in the last case when determinations were made only during the two weeks following lipocaic administration. The 12 depancreatized animals on adequate insulin and lipocaic therapy received

an average of 30 units of regular insulin daily and this limited the glucose excretion to an average of 8 grams in 24 hours. The preparation of lipocaic employed was prepared as indicated in previous reports (1) and consisted essentially of a fat free alkaline alcohol extract of pancreas residue remaining after the removal of insulin. The extract was given by mouth in amounts found adequate to prevent the development of fatty infiltration of the liver and the other manifestations of lipocaic insufficiency (1). With this treatment the blood lipids remained within the normal range and the ketones averaged 5.7 mgm. per 100 cc. In the second group of 5 animals the glucose excretion was adequately controlled with insulin but no lipocaic was given. These animals accordingly developed fatty infiltration of the liver, hypolipemia, and the other signs of lipocaic deficiency. The blood ketones however remained at a low level indicating that hyper-ketonemia is thus not a part of the picture of lipocaic deficiency. The third group of 13 depancreatized dogs was given adequate amounts of lipocaic but insufficient insulin as indicated by the large excretion of glucose in the urine. Under these conditions the average of the blood ketones was relatively high being 21.0 mgm. per 100 cc. This suggests that insulin exerts a great deal more influence on the level of the blood ketones than does lipocaic. Presumably under the conditions of insulin deficiency there occurs an overproduction of ketones in the liver at least when the supply of lipocaic is adequate. The data secured on the fourth group of 20 animals is especially interesting in this connection. These animals were developing the fatty livers of lipocaic deficiency and during this period received inadequate amounts of insulin. The level of the blood ketones averaged 14.7 mgm. which, while somewhat lower than that of the group that received lipocaic, was definitely higher than in the first two groups where adequate insulin was given. It is thus necessary to conclude that an overproduction of ketones can occur in the diabetic liver with insulin deficiency even though no lipocaic is available. The somewhat lower level may be explained as due to the disturbance in liver function as a result of the fatty infiltration so that the overproduction of both ketones and sugar is somewhat interfered with. The last group of 2 depancreatized animals provides final evidence that lipocaic does not significantly influence the level of the blood ketones. These animals developed markedly fatty livers as a result of lipocaic deprivation. They were then given a curative dose of lipocaic and at the same time adequate insulin to control the glucose excretion. The blood ketones remained at the low level of 5.7 mgm. per 100 cc. even though the fat was being cleared from the liver, the blood lipids were rising to the normal level, and large amounts of glucose were being made available. These findings either mean that this glucose which appears when lipocaic is given does not come from the fatty acids in the liver or that ketones are not normal intermediary metab-

olites in this conversion. It is of course possible that the glucose may come from other sources. The improvement in liver function secured by the administration of lipocaic and the disappearance of the excessive accumulation of fat may permit the liver to resume the overproduction of glucose characteristic of the diabetic state.

CONCLUSIONS

1. The blood ketones of depancreatized dogs are not increased during lipocaic deficiency as is the case during insulin deficiency.

2. The administration of lipocaic does not prevent or cure the hyperketonemia of insulin deficiency.

3. The removal of fat from the liver produced by the administration of lipocaic is not accompanied by an increase in the blood ketones.

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EFFECT OF PANCREATIC ACHYLIA ON VITAMIN K ABSORPTION AND PROTHROMBIN TIME

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Investigations have demonstrated a variety of abnormalities dependent upon inadequate digestion and absorption of fats and proteins in the absence of pancreatic enzymes. Boldyreff (1) and Turcatti (2) noted reduction in coagulability of the blood of animals subjected to partial or complete ablation of the pancreas. Ferrari and Cortese (3) corroborated this finding and reported a corresponding reduction in the thrombin content of the blood. The present studies were designed to investigate a possible mechanism of these changes. It has been repeatedly demonstrated (4) that any procedure interfering with the absorption of fats or reducing the functioning capacity of the liver is followed by a vitamin K deficiency and a diminution of the blood prothrombin. It seemed possible that pancreatic achylia might affect clotting of the blood by hindering absorption of the fat soluble coagulating vitamin.

MATERIALS AND METHODS. *Animals.* Adult male and female cats were divided into 3 groups. The first group was subjected to section of the pancreatic ducts and complete isolation of the head of the pancreas from the duodenum. In the second group total pancreatectomy, and in the third group partial pancreatectomy, were performed.

The animals whose ducts were sectioned and the control cats were given 40 grams fresh liver, 5 cc. cod liver oil, a pint of milk and a pint of stew containing vegetables, rice, meat scraps and fish. The second and third groups of animals received the following in single daily feedings: raw lean meat, 150 grams; raw liver, 40 grams; skimmed milk, 180 grams; glucose, 23 grams; sodium chloride, 3 grams; white bread, 25 grams; cod liver oil, 5 cc.; rice bran concentrate,[1] 2 cc. The state of nutrition and general health of the animals were better maintained on the latter regimen. The cats subjected only to section of the pancreatic ducts required no insulin. Only 2 cats survived complete pancreatectomy and their insulin requirements

[1] The rice bran concentrate was kindly furnished by Vitab Products Inc., Emeryville, California. It contained 50 international units vitamin B_1 and 17 Sherman-Bouquin units of vitamin B_2 per cubic centimeter.

were extremely labile. In a few of the cats partial pancreatectomy produced mild diabetes. The largest daily dose of insulin for one of this group was 5 units, but glycosuria was occasionally observed.

Operations. The operations were performed aseptically. Anesthesia was maintained with nembutal when the ducts were sectioned, with open drop ether when pancreatectomy was performed. The anatomical relations of the pancreas and pancreatic ducts of the cat are well described by Heuer (5). The main pancreatic duct was identified with certainty in 7 cats and cut between silk ligatures. In most of these animals the accessory duct was also recognized and similarly sectioned, but in all of this group the head of the pancreas was completely separated from the duodenum for a distance of 1 cm. cephalad and 2 cm. caudad to the level at which the main pancreatic duct enters the duodenum.

In order to control the effect of operative procedure alone on blood coagulation, celiotomy was performed on 2 cats, the tissues in the region of the pancreas and biliary passages being handled but not otherwise altered.

Six cats were subjected to partial pancreatectomy. The entire head of the pancreas and the proximal portion of the splenic limb were removed without injury to the pancreatico-duodenal artery and vein and common bile duct. The distal extremity of the tail of the pancreas, about 2 to 3 cm. long, was left in the mesentery with its blood supply intact. It had no connection with the gastro-intestinal tract. Histologic sections of the resected tissues showed normal pancreas.

Complete pancreatectomy was conducted in two stages, the greater part of the pancreas being excised at the first operation. One week later the remaining segment was resected.

Total lipid analysis of stool specimens and livers. Specimens and tissues were desiccated to constant weight and extracted over steam with 95 per cent alcohol and 2 changes of chloroform in turn. The extracts were freed of solvent and the residue reëxtracted 3 times with petroleum ether. The petroleum ether soluble material was recorded as per cent of dry weight of original material.

Determination of prothrombin time. Thromboplastin solution was prepared from blood-free chicken brains by maceration and repeated dehydration with absolute acetone. Freed of solvent, the powder was either used promptly or stored under nitrogen at −70°C. Before using, 0.3 gram was added to 5 cc. 0.9 per cent saline and 0.1 cc. 3 per cent sodium citrate. The mixture was incubated at 45°C. for 10 minutes and the larger particles removed by centrifugation.

Blood was obtained for determination of prothrombin time either from the femoral vein or by heart puncture. Determinations conducted simultaneously on samples from vein and heart checked within the range of experimental error. Immediately on withdrawal, 2 cc. whole blood were

mixed with 0.167 cc. of 3 per cent sodium citrate. The plasma was separated by centrifugation for 30 minutes at 1800 R.P.M. and kept about 1½ hours before the prothrombin time was determined. No measurable change occurred during this time. During the entire period between withdrawal of the blood and determination of prothrombin time, the materials were surrounded by ice water and centrifugation was conducted by means of an ice chamber and chilled cups.

Prothrombin time was determined by means of the coagelometer as described by Baldes and Nygaard (6). The portions of the apparatus in contact with solutions were immersed in a constant temperature bath regulated to 40° ± 0.1°C. Before prothrombin time was determined, each specimen of plasma was placed in the bath for 5 minutes. Four-tenths cubic centimeter of a mixture containing equal parts of calcium chloride solution (1.11 grams anhydrous $CaCl_2$ in 100 cc. H_2O) and thromboplastin solution were added to 0.2 cc. of the plasma in the glass tube and speedily placed in the coagelometer. The formation of a fibrin network in the plasma reduced the intensity of the beam of light incident upon the photoelectric cell. The prothrombin time was defined as the interval between mixture of the solutions and the sudden downward deflection of the microammeter needle that marked the instant of coagulation.

Hematocrit determination. Viscosity of the blood is known to affect the velocity of coagulation. Any possible variations in viscosity attendant upon anemia or dehydration were controlled by hematocrit determinations on the specimen obtained for determination of prothrombin time.

Whole blood clotting time. A few drops of blood from the ear were collected in a small tube and shaken in a water bath at 37°C. until it no longer flowed on light tapping.

Bleeding time. The period of flow of blood from the ear prick was recorded.

Assay of vitamin K content of liver. Only those livers obtained immediately after death were tested. The liver was cut to a pulp, frozen at −70°C., and dried in high vacuum. The desiccated liver was pulverized and packed into gelatin capsules, each containing about 70 mgm. For each test, 30 one-day old white leghorn chicks were placed directly on the vitamin K free diet described by Ansbacher (7) and designated as ration K-1. The diet was administered for 14 to 17 days, depending on the rapidity of development of the deficiency state. The chicks were divided into 3 groups and the average clotting time for each group of 7 to 10 chicks determined. If the blood failed to clot in 30 minutes, it was discarded and the clotting time regarded as 30 minutes in determination of average time. After receiving 1, 2 or 3 capsules of the powdered liver, the chicks were deprived of food for 20 to 24 hours when the clotting time was again deter-

mined. The curative effect of the liver was roughly estimated as 0 to ++++ depending on the amount of alteration in clotting time occurring during this one test period.

Necropsies. Complete morphologic studies were conducted on all animals which died spontaneously or were killed by intravenous injection of air.

RESULTS. *Total fat content of stools.* Figure 1 shows the abrupt and persistent rise in total fat extracted from random stool specimens after section of the pancreatic ducts and separation of the head of the pancreas from the duodenum. Only one cat, no. 7, showed a gradual decrease in

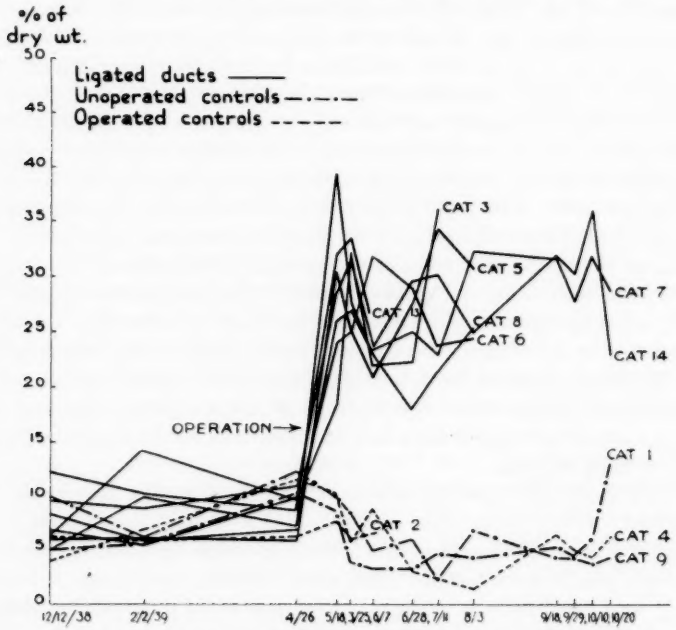

Fig. 1. Total fat content of stools following section pancreatic ducts

stool fat during the next six months not included in the graph. A value of 9.2 per cent was obtained shortly before the animal was sacrificed. Figure 2 shows that pancreatectomy, either complete or subtotal, was followed by an immediate and striking rise in stool fat from a normal of about 5 per cent to values ranging from 14 to 46 per cent dry weight.

Prothrombin time. This value was calculated as the mean of duplicate determinations which rarely differed by more than one second. The normal value for each set of determinations was the mean of the prothrombin times of the control animals for that day. This value varied considerably

from one series of determinations to another. The percentage deviation from the normal was calculated for each prothrombin time and expressed as a plus or minus value according as the time was greater or less than that of the control cats for that day. The relationship of the prothrombin times of the experimental animals as a group, to the normal prothrombin times of the control group, was found by calculating the net mean deviation from normal of the respective groups as follows. The difference between each prothrombin time and the normal for that series of determinations was found and expressed as a plus or minus value. These values for a given

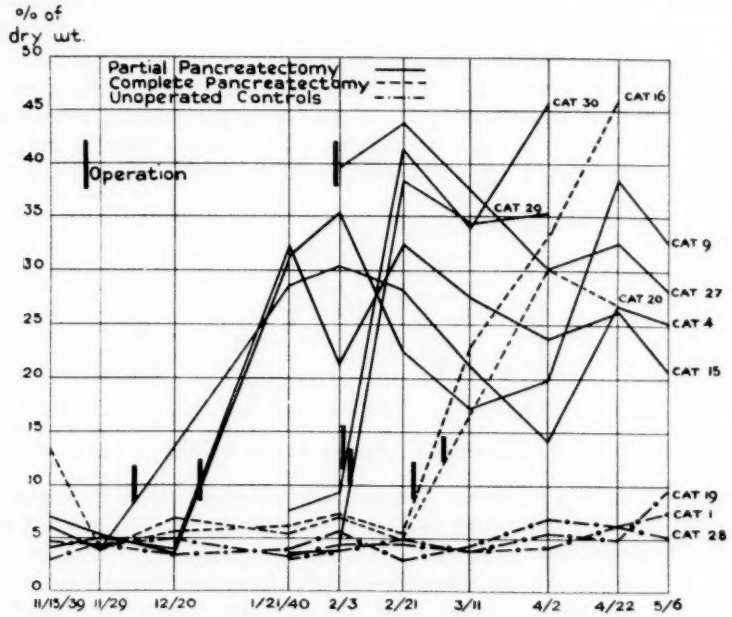

Fig. 2. Total fat content of stools following pancreatectomy, partial and complete

group of animals were added and the net sum was divided by the number of cats in the group. This quotient indicated how many seconds greater or less than normal the average prothrombin time of a given group of animals was found to be and was called the *net mean deviation from normal* of this group. It was necessary to express this as percentage deviation above or below normal in order that the values obtained on different days could be compared. Since the normal value was calculated as the mean of the prothrombin times of the control animals, the net mean deviation of this group was always zero.

During the $3\frac{1}{2}$ month preoperative period the deviation from normal

of most animals fell within the range of −10 to +10. The greatest variation between individual reading was −26 to +34. The 2½ month period immediately following section of the ducts presented a complicated picture with fluctuations between −48 and +116. Five months after operation, 6 of the 7 cats were dead. Two of these 6 were showing progressive increase of the prothrombin time reaching 105 per cent and 57 per cent above normal during the final week of life. The values of 3 others varied widely, but the terminal readings (72 per cent, 116 per cent and 26 per cent above normal respectively) were significantly higher than the preoperative level. A single animal was sacrificed after 13 months. Seven months after operation the prothrombin time of this animal reached a maximum value of 64 per cent above normal from which it fell slowly to the terminal value of +10 per cent. The stool fat content was also restored to a normal level.

Although there was considerable variation of the prothrombin times both before and after operation, if the animals with ducts sectioned be considered as a group (fig. 3), the average prothrombin time was greater than at

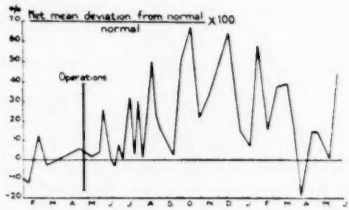

Fig. 3. Prothrombin times following section pancreatic ducts

any time before operation and the average value did not fall to or below normal. From October 1939 to June 1940 the curve was determined by the prothrombin times of a single cat (no. 7).

The 6 cats in which the greater part of the pancreas had been removed also differed in their response. For the 7 months before operation the majority of the prothrombin times for the individual animals fell within a comparatively narrow range and the values greater than normal were about equaled in number by those less than normal. After operation the positive deviations from normal were greater and the negative deviations, which were part of the normal picture, were negligible. The very marked prolongation of prothrombin time of a single animal was progressive until it was 293 per cent above normal when he bled to death. The prothrombin values obtained from one cat did not change appreciably.

This group as a whole (fig. 4) showed a greater prolongation of prothrombin time after operation than did the group of cats whose ducts were sectioned.

Hematocrit determinations. Alterations in hematocrit values occasionally

occurred as individual animals developed anemia or dehydration, but there was no correlation between changes in viscosity of the blood and the prothrombin times.

Whole blood clotting times. The coagulation times obtained from cats whose ducts were sectioned were only slightly higher than those of the controls. The highest value obtained was 9 minutes. The whole blood clotting times of cats completely or partially deprived of pancreatic tissue showed individual variation. The blood of one cat whose prothrombin time was greatly prolonged, failed to clot under 19 minutes shortly before the animal died from protracted hemorrhage. The other animals showed a rise at some time to approximately 10 minutes but the elevation was not a progressive or persistent one. The cat maintaining a normal prothrombin time failed to show any prolongation of clotting time.

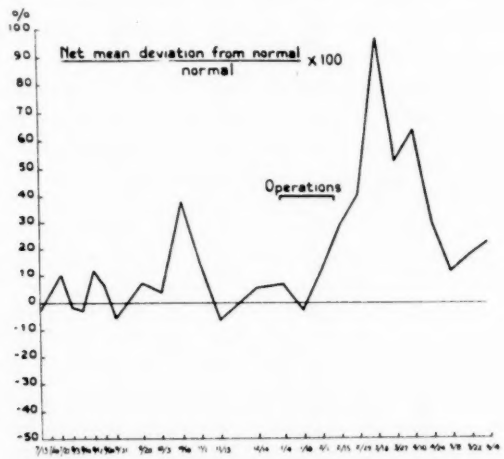

Fig. 4. Prothrombin times following partial pancreatectomy

Bleeding time. There was no significant rise in bleeding time following section of the pancreatic ducts. After pancreatectomy, partial and complete, it was only slightly altered. In most instances there was a rise to 4 or 5 minutes from a normal of 1 to 2 minutes. The cat showing the greatest prothrombin time continued to bleed for 8 minutes on one occasion and the last measure recorded on another cat was similarly elevated. Both cats on whom complete pancreatectomy had been performed evinced moderate prolongation of bleeding time and one bled for several hours shortly before death.

Vitamin K assays of cat livers. Normal desiccated liver has a curative effect when fed to chicks deficient in vitamin K. The data presented in table 1 indicate that section of the pancreatic ducts usually results in diminu-

tion of vitamin K content of the liver. In 3 instances when the cats had died within 3½ months after the operation at least 140 mgm. of dry liver

TABLE 1

Vitamin K assay cat livers

CAT NO.	LENGTH LIFE AFTER OPER.	NO. TEST CHICKS BEFORE	AV. CLOT. TIME BEFORE	NO. CAPS. DRIED LIVER FED*	NO. TEST CHICKS AFTER	AV. CLOT. TIME AFTER†	VITAMIN K CONTENT OF TEST DOSE
I. Unoperated controls							
	months		*minutes*			*minutes*	
1		11	28	1	8	7	+++
		9	28	2	8	1½	++++
		7	>30	3	6	2	++++
19		10	26	1	10	4	++++
		9	27	2	7	2½	++++
		9	29	3	9	3	++++
28		10	27½	1	8	10	+++
		10	28	2	10	4½	++++
		10	>30	3	9	2	++++
II. Section of pancreatic ducts							
13	1	7	27	1	5	25	0
		7	>30	2	5	16	++
		8	26	3	8	15	++
3	2	9	29	1	9	17	++
		9	29	2	8	12	+++
6	3	10	28	1	8	27	0
		10	26	2	7	26	0
		11	29	3	8	23	0
8	3½	10	>30	1	9	28	0
		9	28	2	9	20	+
		9	29	3	7	16	++
14	5	10	27	1	10	25	0
		11	28	2	8	26	0
		9	29	3	7	27	0
7	13	9	27	1	7	9	+++
		9	28	2	7	3	++++
		9	>30	3	7	2	++++

* One capsule = about 70 mgm. dried liver.

† Taken 24 hours after test dose.

were required to alter the coagulation time of the deficient chicks and even the maximum dose of about 200 mgm. failed to restore the normal clotting

time. There was no curative effect in the test amounts of liver obtained from 1 cat living 3 months and another living 5 months after operation. Cat 7, whose post operative period extended beyond a year, had at least a normal amount of the vitamin in the liver. This is in accordance with the restoration of stool fat content and prothrombin time to normal levels during the latter part of its course.

A more pronounced deficiency in vitamin K content of the liver followed pancreatectomy, either partial or complete (table 2). The liver of 1 animal had a slight curative effect; the others had no effect in the dose used. The liver lipids following partial pancreatectomy were elevated only in 2 cats (table 3) while the vitamin K content of the liver was reduced in all.

Hemorrhagic manifestations of a deficiency state. The animals were observed for the appearance of spontaneous hemorrhages. None was detected in skin, mucous membranes or conjunctivae. Two fatalities were due to hemorrhage initiated by cardiac puncture at the time when the prothrombin times were at their height. One cat, whose pancreas was partially resected, died following protracted hemorrhage into the intestinal tract. Four of the six cats subjected to partial pancreatectomy had gross hematuria at intervals and red cells could be found in the urine on other occasions. Urine analyses were rarely performed when the ducts alone were sectioned and no blood appeared in these occasional specimens.

Necropsies. Histologic study of the organs revealed the following facts. The pancreas was atrophic and showed a varying degree of fibrosis on every occasion but one. One cat, whose prothrombin time and stool fat were little elevated at the time he was sacrificed 13 months after operation, showed only moderate atrophy of the pancreatic acini. The amount of fat present in the liver was consistent with the values obtained by chemical analysis. There were no other significant changes in the liver cells. The mucosa of the alimentary tract was studded with petechial hemorrhages in three cats. The animal suffering a profound intestinal hemorrhage had an acute ulcer of the duodenum.

Three cats in which the ducts had been sectioned and all those with partial or complete pancreatectomy showed renal changes. They varied from an accumulation of protein precipitate in the glomerular spaces of Bowman and lumina of the tubules to scattered areas of necrosis of the epithelium lining convoluted tubules. Occasionally red cells and casts were also found in the tubules. The kidneys of the one cat surviving 13 months were reduced in size and showed atrophy of tubules with fibrosis of the interstitial tissues. In many areas the convoluted tubules had disappeared entirely. The better preserved tubules were dilated, contained hyaline casts and their lining epithelium appeared to have been regenerated. Only a few plasma cells were present. The glomeruli and blood vessels were normal.

Discussion. The establishment of pancreatic achylia by either partial

pancreatectomy or section of the pancreatic ducts is followed by a variable but significant prolongation of prothrombin time in the cat. Pronounced reduction of prothrombin, comparable to that initiated by isolation of the

TABLE 2

Vitamin K assay cat livers

LIVER OF CAT NO.	LENGTH LIFE AFTER OPER.	NO. TEST CHICKS BEFORE	AV. CLOT. TIME BEFORE	NO. CAPS. DRIED LIVER FED*	NO. TEST CHICKS AFTER	AV. CLOT. TIME AFTER†	VITAMIN K CONTENT OF TEST DOSE
I. Complete pancreatectomy							
	months		*minutes*			*minutes*	
16	2	8	28	1	7	26	0
		9	29	2	9	26	0
		10	>30	3	10	28	0
20	2	9	29	1	8	26	0
		8	>30	2	7	28	0
		8	>30	3	6	29	0
II. Partial pancreatectomy							
29	2	9	29	1	8	28	0
		8	29	2	6	30	0
		8	28	3	7	27	0
30	2	7	27	1	5	29	0
		8	29	2	8	28	0
		8	27	3	6	29	0
4	3	10	28	1	8	28	0
		8	27	2	6	29	0
		7	29	3	6	28	0
9	3	9	>30	1	7	29	0
		10	29½	2	9	27	0
		10	27½	3	8	26	0
15	3	9	27	1	8	25	0
		9	>30	2	7	29	0
		9	28	3	8	29	0
27	3	7	28	1	6	26	0
		7	>30	2	7	29	0
		7	28	3	7	20	+

* One capsule—about 70 mgm. dried liver.

† Taken 24 hours after test dose.

biliary tract from the intestine, is less often observed. Spontaneous hemorrhage occurs more frequently in cases of biliary obstruction than in pancreatic deficiency, but it is recognized that the prothrombin level must

be lowered to approximately 20 per cent of the normal before hemorrhages appear.

When the ducts alone are sectioned and the organ is allowed to remain in the body, there is less deviation from normal of the prothrombin times and of the quantity of lipids in the stools. Although this procedure is soon followed by atrophy of the acini, the islets of Langerhans are undisturbed. It is possible that an internal secretion such as the lipocaic discussed by Dragstedt (8) is responsible for the difference in effect of extirpation of the pancreas from that of mere atrophy and fibrosis of this organ.

A rough parallel can be drawn between the degree of altered elimination of lipids in the stools and the elevation of prothrombin times in the experimental animals. Obviously the examination of random stool specimens without complete study of fat intake and output for extended periods does not permit an absolute comparison between fat metabolism and prothrombin content of the blood. However, both the stool lipids and the prothrombin times were more strikingly elevated in the early post operative period and tended toward a gradual restoration of the normal value. This was especially true of the prothrombin values. The explanation may lie in the known sources of lipase other than the pancreas. In the absence of the external secretion of this organ, fat splitting enzymes of stomach, intestine and even of the intestinal flora may play an increasingly prominent rôle in digestion.

TABLE 3

Total lipid content of liver

Per cent of dry weight

EXPERIMENTAL CONTROLS		SECTION OF PANCREATIC DUCTS		PARTIAL PANCREATECTOMY	
Cat no.	Per cent lipid	Cat no.	Per cent lipid	Cat no.	Per cent lipid
Unoperated controls		3	15.5	4	12.8
1	12.0	6	14.9	9	45.5
19	19.8	8	19.7	15	23.6
28	14.1	13	13.2	27	14.3
Operated control		14	14.8	29	15.2
2	13.7	Complete pancreatectomy		30	16.2
		16	12.4		
		20	14.6		

The demonstration of a reduction of vitamin K content of the liver in animals with pancreatic achylia provides strong indication that a deficiency in the fat-soluble vitamin due to faulty fat digestion is the mechanism by which blood clotting is delayed. In fact, diminution of the vitamin K content of the one organ tested was a more constant finding than pronounced elevation of prothrombin time. Undoubtedly stores of the vitamin are not

absolutely depleted and it is possible that a critical level must be reached before the deficiency is reflected in coagulation defects.

The reduction of vitamin K in the liver is independent of the amount of fat in this organ. Fatty livers were not the rule in the pancreatectomized cats despite obvious disturbances in fat metabolism as demonstrated by stool analyses. When the ducts alone were sectioned, an adequate amount of choline may have been obtained from the internal pancreatic secretions to maintain a normal phospholipid turnover in the liver. The cats subjected to partial and complete pancreatectomy were given a high protein diet containing sufficient quantities of methionine to perform a similar function in most instances.

An explanation for the occurrence of hematuria and necrosis of renal epithelium is suggested by the experiments of Griffith and Wade (9) and more recently by Györgi and Goldblatt (10). Administration to rats of a diet deficient in choline resulted in tubular necrosis and interstitial hemorrhage in the kidneys. Since pancreatectomy deprives the body of at least a part of its choline such a mechanism may have been responsible for the renal damage in our cats.

SUMMARY

Adult cats were deprived of the external secretion of the pancreas by section of the pancreatic ducts, complete extirpation of the pancreas or by subtotal pancreatectomy. In every instance the operations were followed by a marked rise in the lipid content of the stool.

There was considerable fluctuation of the prothrombin times but the average prothrombin time following section of the ducts surpassed that observed before operation and did not return to normal. The effect of pancreatectomy was more striking but equally variable.

Reduction of prothrombin caused only moderate alteration of whole blood clotting time and slight prolongation of bleeding time.

The vitamin K content of the liver, as demonstrated by biological assay, was reduced after duct transection and even more strikingly lowered when the greater part of the pancreas was resected. This could not be accounted for by accumulation of fat in the liver since fatty liver rarely occurred.

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THE EXCHANGE OF RADIOACTIVE POTASSIUM WITH BODY POTASSIUM

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This paper represents a continuation of previous work in which radioactive potassium was used to study the permeability of various tissues of the rat (Noonan et al., 1941). It was found in general that after injection into a rat radioactive potassium was absorbed most rapidly by the viscera and more slowly by muscle, nerve and skin. In the first 2 hours after injection the remarkable fact was observed that in relation to the amount of total potassium present there was more radioactive potassium in many visceral organs, particularly the liver, than there was in the plasma. It was chiefly for the purpose of furthering the investigation of this fact that more experiments on the rabbit and other animals were undertaken. Larger animals offer the advantage that successive samples of blood and liver can be taken from the same animal.

METHODS. Details of the methods used have already been explained. It need only be stated that samples of tissue were dissolved in nitric acid and the digest was analyzed in duplicate for total potassium and used for the determination of radioactive potassium of the sample by a Bale immersion type of Geiger-Mueller counter.

The nomenclature which is used is given in the following equations:

$$\text{Radioactivity} = \text{RA} = \frac{\text{counts per kgm. wet weight of sample}}{\text{counts injected per kgm. body weight}}$$

$$\text{Potassium radioactivity} = \text{KRA} = \frac{100 \times \text{RA}}{\text{mM of K per kgm. of sample}}$$

$$\text{Relative KRA} = \frac{\text{KRA of tissue}}{\text{KRA of plasma}}$$

(Relative KRA) $\times$ 100 = per cent penetration or per cent exchange of K^* with K^+.

1. Rabbit experiments. The fate of injected radioactive potassium in the whole animal is illustrated by the experiment in table 1 in which the whole animal was killed 15 hours after injection of radioactive potassium and was

divided into four parts; skin, muscle, viscera and carcass. Plasma samples were also taken in order that the relative KRA could be calculated. The results show that 54 per cent of radioactive material is recovered in the muscle in which 55 per cent of the total potassium of the body is located. In general the distribution in all 4 groups of the tissues is just proportional to the amount of potassium which they contain. In all the tissues about 85 per cent of the potassium has exchanged with the radioactive material. After shorter periods the viscera contained relatively more of radioactive potassium and the muscles relatively less. The total potassium of the body is about 72.9 mM per kgm. according to these figures. The amount of total potassium exchanged can also be calculated from the expression $\frac{100}{\text{KRA of plasma}} = \frac{100}{2.33}$ or 43 mM per kgm. or 59 per cent of the total. There is, therefore, some discrepancy in the figures, for the potassium ex-

TABLE 1

Fate of injected radioactive potassium in the rabbit after 15 hours

	WEIGHT		POTASSIUM			TOTAL COUNTS		RA	KRA	RELATIVE KRA
	Grams	In per cent of actual	mM/ kgm.	Total mM	Per cent of total	Counts $\times 10^{-2}$	In per cent of injected			
Skin	323	13.5	34.8	11.3	7.1	406	8.1	0.58	1.67	0.86
Muscle	828	34.5	105.6	87.3	55.4	2715	54.3	1.58	1.56	0.80
Viscera	458	19.1	61.6	28.2	17.9	968	19.4	1.01	1.64	0.85
Carcass	554	23.1	55.8	30.9	19.6	1270	25.4	1.05	1.88	0.87
Total	2163	90.2	72.9	157.7	100	5359	107.2			
Actual	(2400)	(100)				(5000)	(100)			

change as calculated from tissues analysis was over 80 per cent complete. A probable explanation of these figures will be considered later.

The results of analyses of the tissues from 9 different rabbits are given in table 2. We have listed only the potassium contents (K) and the radioactivity of the samples (RA). Since the plasma values are given in each case it is possible to calculate KRA and the relative KRA or the per cent penetration. These last values are plotted in figures 1 to 4. It is evident that the rat experiments are in general confirmed. Diaphragm, heart, kidney, liver and gastro-intestinal tract give values greater than 1 during the first 1 to 3 hours. Brain, nerve, muscle, testis, skin, bone marrow and bone show slower penetration without an initial peak as in the case of rats. The 24 hour point on the skin curve in figure 2 appears to be erroneously high since it should not be much if any greater than 1.0. The figures for bone are calculated on the assumption that bone contains 0.1 per cent potassium. We are indebted to Dr. H. C. Hodge for this tentative figure

based largely on a critical selection from the varied figures in the literature (cf. Steadman, Hodge and Horn, 1941).

It follows from the definition of the tissue radioactivity (RA) that (RA of sample) × weight of sample in per cent of body weight = per cent of injected dose found in sample.

Utilizing this relationship and the weights of rabbit tissues in per cent of the body weight the distribution of the injected dose may be calculated from the data of table 2. Thus it appears that the heart, lung, kidney, diaphragm, liver and gastro-intestinal tract together comprise only 12 to 13

TABLE 2

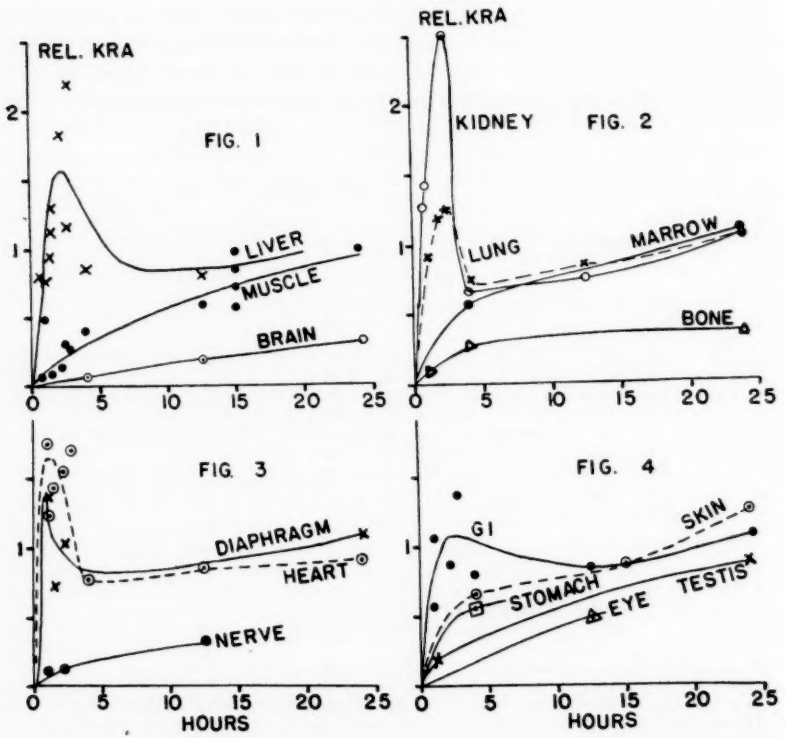
Potassium content (K) and radioactivity (RA) of rabbit tissues

EXPERIMENT	TIME	DOSE	INJECTION	PLASMA		LIVER		MUSCLE		HEART		INTESTINE		DIAPHRAGM		LUNG	
				K	RA	K	RA	K	RA	K	RA	K	RA	K	RA	K	RA
	hours	*m.eq./kgm.*															
1	24	0.92	SC	4.59	0.107	77.6	1.91	117	2.72	86.8	1.87	90.3	2.34	98.0	2.48	82.4	2.02
2	12.5	0.52	SC	5.98	0.15	93.4	1.92	143	2.14	96.2	2.08	104	2.21			98.6	2.07
3	4.0	0.31	SC	3.75	0.106	79.8	1.93	110	1.24	76.6	1.67	79.8	1.79	90.4	2.11	76.0	1.58
4	1.0	0.62	SC	5.32	0.11	80.8	1.88	119	.72	79.5	2.77	90.0	1.91	94.3	2.6	80.8	2.02
5	1.0	1.2	SC	7.26	0.22	81.0	1.88	103	1.5	76.8	2.89	86.4	1.48	99.0	3.33	92.2	2.50
6	2.8	0.44	Vein	5.58	0.21	74.7	6.06	119	0.81	89.3	5.6	99.4	5.09				
7	2.2	1.91	Vein	5.72	0.16	71.6	3.65	138	0.58	97.3	4.3	107	2.67	95.5	2.91	88.6	3.01
8	1.6	0.76	Vein	4.87	0.13	91.4	2.40			99.3	3.84			94.5	1.85		
9	1.45	0.36	Art.	13.8	0.43	76.9	2.7	105	0.29								
				NERVE		KIDNEY		BRAIN		SKIN		TESTIS		MARROW		BONE	
1	24					60.4	1.51	87.4	0.69	31.8	0.98	98.5	2.13	60.4	1.57	25.6	0.23
2	12.5			75.5	0.63	81.6	1.54	86.4	0.40								
3	4.0			42.4	0	56.2	1.10	94.6	0.17	25.6	0.49			60.4	0.79	25.6	0.20
4	1.0			43.2	0.097	81.0	2.32					103	0.41				
7	2.2			49.8	0.19												

K = potassium content in m.eq. per kgm. wet. RA = radioactivity (see text). Additional data for K and RA and relative KRA are respectively in the various experiments: expt. 1, stomach muscle, 102, 2.62 and 1.10; stomach mucosa, 84.1, 1.97 and 1.01; expt. 2, gall bladder and contents 24.6, 0.60 and 0.96; eye, 24.5, 0.31 and 0.50; expt. 3, stomach 78.8, 1.25 and 0.56.

per cent of the body weight and contain 12 to 15 per cent of the body potassium, but after the first hour they account for 24 per cent of the injected counts. The liver itself being 5 per cent of the body weight and containing 5 per cent of the body K takes 10 per cent of the injected dose in the same time. Conversely, if the muscles comprise 40 per cent of the body weight the RA of muscles cannot be greater than $\frac{100}{40} = 2.5$ unless the muscles contain more radioactive material than was injected. For this reason the high value of 2.72 for the RA of muscle in the 24 hour experiment (table 2) appears to be erroneous. With this exception the highest

RA found in muscles is 1.98 after 12.5 hours. In another 15 hour experiment (table 1) the pooled muscles gave an RA of 1.58 while the values for individual muscles were as follows: rectus femoris, 1.55; gastrocnemius, 1.63; triceps, 1.94; masseter, 2.12; longissimus dorsi, 1.20; rectus abdominis,



Figs. 1–4. Relative potassium radioactivity (rel. KRA) plotted against time in hours.

Shows the penetration of radioactive potassium into various rabbit tissues as a function of time. Complete exchange is indicated by a value of relative KRA = 1.0. In figure 2 the bone curve includes one point at 1 hour from a similar cat experiment (table 3, no. 3). One rabbit point at 1.6 hours with a rel. KRA = 1.12 was discarded as erroneous for unknown reasons.

1.35; psoas, 1.41; and gluteus 1.62. On the average therefore, the muscles at 15 hours accounted for 62 to 80 per cent of the injected dose assuming the muscles equal to 40 to 50 per cent of the body weight.

2. Cat experiments. Three experiments on cats are summarized in table 3. Detailed figures are omitted and only the per cent penetration as cal-

culated from analyses in both plasma and tissues are included. As in rabbit and rat, the visceral organs show values greater than 100 per cent in short time experiments. After longer times all tissues would show approximately 100 per cent penetration. The penetration of muscle is particularly low in these experiments, not greater than 14 per cent. The total potassium exchanged is only 39 mM per kgm. after 2.7 hours. The total potassium was determined in one cat by dissolving the whole animal in nitric acid and was found to be 77.6 mM/kgm. Therefore, about one-half of the potassium of the animal had exchanged in 2.7 hours. After only one hour the exchange was less than 17 per cent and the plasma KRA values were correspondingly high (expts. 2 and 3), but this may have been due to a poor circulation from previous loss of blood in these 2 animals (see legend table 3). The rapid penetration into cat erythrocytes is also illustrated in this table and is due chiefly to the small potassium content of

TABLE 3

Penetration of potassium into cat tissues

CAT NUMBER	WEIGHT OF CAT	DOSE OF K	INJECTION	TIME	PLASMA KRA	EXCHANGED POTASSIUM	LIVER	HEART	KIDNEY	INTESTINE	DIAPHRAGM	BONE MARROW	BONE	MUSCLE	NERVE	ERYTHROCYTES
	kgm.	*m.eq./kgm.*		*hrs.*												
1	2.3	0.29	Arterial	2.7	2.56	39	135	118	117					13.5	25.4	30
2	2.1	2.46	Vein	1.0	6.0	16.6	123	155		123	48	47		14		40
3	2.2	0.29	Vein	1.0	12.8	7.8	98	151	125	80			10	9		65

All cats under dial-urethane anaesthesia. Cats 2 and 3 had previously lost about 20 cc. of blood and had received injections of liquoide-Roche to prevent clotting. This accounts perhaps for the poor exchange.

these cells (cf. Mullins et al., 1941). The slower penetration of the muscle on the other hand must be dependent on a smaller permeability to potassium or to a poor circulation through the muscle as compared to the liver.

3. Frog experiments. A number of observations have been made on frogs which are summarized in figure 5. In this case we have plotted not the relative potassium activity but the potassium activity (KRA) or the ratio of RA to total potassium. The values of the relative KRA or fractional penetration are given in table 4. The radioactive material was injected into the dorsal lymph sac. Plasma was obtained by syringe from the aorta with the use of heparin as an anticoagulant. The frog was previously immobilized by destroying the brain.

High values of KRA in the plasma were obtained after one hour but they fell rapidly to an equilibrium value of about 1.5 representing 67 mM

per kgm. of total potassium exchanged as indicated in figure 5c. Potassium in the heart exchanges rapidly with the potassium in the serum and the heart curve lags slightly behind the plasma curve. Other tissues lag to greater degrees but many of them such as lung, skin and kidney show slight peaks corresponding to the high plasma values in the initial period. Muscle, ovary and red cells are particularly slow. But with the exception of the red cells and the ovary (which presumably has a poor circulation) the

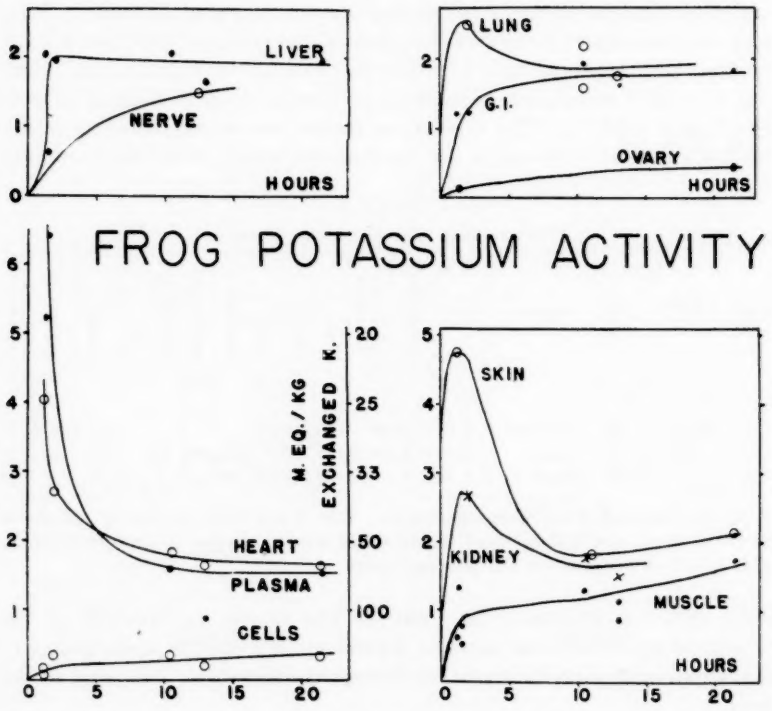


Fig. 5. Values of potassium radioactivity (KRA) plotted against time. A value of 1.5 in the plasma would represent complete penetration or complete exchange. Upper, a and b; lower, c and d.

KRA of all the tissues approximates that of plasma at the end of 20 hours, i.e., their potassium is 100 per cent exchanged in this time. In the frog it is the skin which takes up the potassium most rapidly, probably because it reaches the skin after injection in the dorsal lymph sac in especially high concentration. In one frog the skin accounted for 10 per cent of the body weight but contained 21 per cent of the radioactive material after 1.25 hours at which time 91 per cent of its potassium had exchanged with the

radioactive material. The ovary on the other hand accounted for 20 per cent of the body weight and contained only 1.2 per cent of the injected material. In another 19 hour experiment 20 per cent of the body weight was due to the ovary which held only 2.2 per cent of the radioactivity.

In table 4 are given the average potassium contents of the various tissues analyzed as well as the values of relative KRA or fractional penetration. Under each experiment are also given the plasma KRA and the corresponding total exchanged potassium as well as the total K in the whole animal as found by analysis. The exchanged K in the longer experiments was

TABLE 4

Relative KRA or fractional penetration in various frog tissues

	AVERAGE POTASSIUM	RELATIVE KRA					
		1.25 hours	1.4 hours	2.0 hours	10.5 hours	13.0 hours	21.5 hours
	m.eq./kgm.						
Muscle (gast.)	88.0	0.30	0.086	0.42	0.83	1.0	1.16
Muscle (thigh)	86.1	0.26	0.122		0.82	1.26	1.14
Liver	84.9	0.39	0.099	1.18	1.28	1.82	1.26
Heart	80.1	0.78		1.60	1.14	1.82	1.08
G.I. tract	74.3	0.23		0.74	1.18	1.86	1.19
Skin	47.1	0.91			1.14		1.40
Ovary	46.0	0.026					0.28
Oviduct	22.2						0.91
Erythrocytes	78.1	0.013	0.024	0.19	0.23	0.22	0.21
Kidney	70.8			1.60	1.12	1.70	
Lungs	64.7			1.50	1.18	1.97	
Nerve	95.2					1.69	
Carcass	53.1	0.43			1.38		1.12
Plasma	6.2	1.0	1.0	1.0	1.0	1.0	1.0
Plasma KRA		5.23	6.46	1.68	1.61	0.88(?)	1.54
Exchanged K (m.eq./kgm.)		19.1	15.5	59.5	62.1	113	65
Total K found (m.eq./kgm.)		49			48.3		52

around 60 m.eq. per kgm. while the total found by analysis was around 50 m.eq. The discrepancy is probably due in part at least to some loss in sampling. The plasma KRA in the 13 hour experiment is doubtless too low because the calculated exchanged potassium is impossibly high. Consequently all the fractional penetrations in this experiment are probably about 1.7 times too high. Plasma samples obtainable from a 40 gram frog are so small that duplicate determinations of potassium are impossible; this probably accounts for the error.

The excretion of radioactive K was followed in only 2 of the frog experiments. In one of these (not included in table 4 because incomplete)

2.5 per cent of the dose was found in the water in which the frog was partially immersed for 19 hours. In the 13 hour experiment of table 4, 12 per cent of the dose was excreted and the figures given are calculated on the basis of the counts still retained in the body. After 21 hours (table 4) 87 per cent of the injected counts were recovered from the frog and after $1\frac{1}{4}$ hours, 96 per cent.

4. Potassium in the viscera. In the case of the frog we have seen that the high values of potassium radioactivity or KRA which are found soon after injection are due to similarly high values in the plasma with which the tissues tend to come into equilibrium. It must be emphasized however that the peaks shown in figures 1 to 4 for the visceral organs are peaks in relative KRA, i.e., the KRA of these tissues must be higher than the simultaneous KRA of the plasma. Such a condition might occur in a tissue which is lagging behind the plasma when the plasma KRA is rapidly falling. Indeed in figure 5c the KRA of the heart is for a time higher than that of the plasma. This raises the question whether the high relative KRA in the liver could be similarly explained. The following theoretical considerations lead to the conclusion that values of KRA slightly greater than 1 could be so explained but the very large values of 1.5 or 2.0 are too large for such a mechanism.

Let y and x equal respectively the KRA (K^*/K^+) of the plasma and tissue respectively. The tissue is considered to be small in size compared to the plasma so that y varies independently of x. Then x tends to approach the value of y by exchange of potassium between tissue and plasma and we may write

$$\frac{dx}{dt} = K(y - x) \tag{1}$$

To solve this equation it is necessary to express y in terms of t. For this purpose some experimental values for y were fitted empirically by the equation

$$(y - a) = (y_0 - a)e^{-kt} \tag{2}$$

This equation is plotted as a dotted line in figure 6 where $a = 0.8$ and $k = 0.383$. The experimental points (representing smoothed data on rat plasma — cf. Noonan et al.,1941) are included to show how well the equation fits. This value of y is now substituted in equation (1), which was kindly integrated for us by Prof. H. A. Blair of this department. The integral form of the equation appears in figure 6 and some of the curves representing values of x which are plotted from it using various values of K are also shown. It is evident that x continues to increase until $x = y$ when the two curves cross. Thereafter $x > y$ but continues to approach the value of y at a rate which is proportional to $(y - x)$. When mixing is

finally complete y and x have again the same value. In spite of wide variation in the choice of the constants K and k it was found impossible to make x more than 15 to 20 per cent larger than y at any time.

On the basis of these considerations we feel forced to conclude that the simple process of diffusion and exchange of potassium will not allow much more radioactivity in the tissues per unit of total potassium than is found in the plasma.

It therefore appears that the liver like other visceral organs has the power of taking an excess of potassium out of the plasma and giving it up slowly to other tissues of the body, particularly the muscles. If this excess

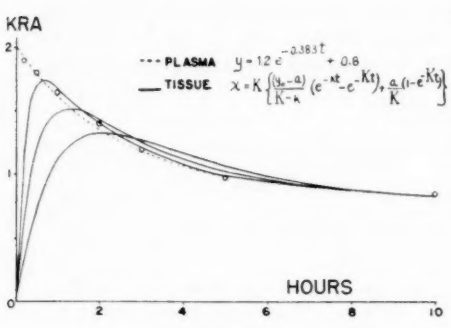


Fig. 6

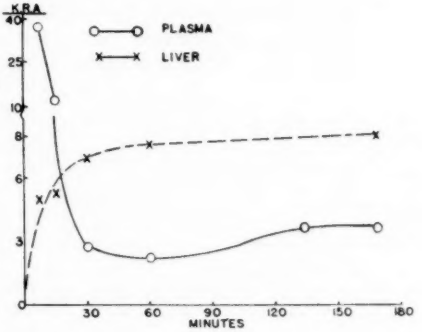


Fig. 7

Fig. 6. Theoretical curves indicating the way in which the KRA of tissues tends to approach the KRA of plasma by the process of exchanging K^{42} for K^{39}. The solid lines are theoretical curves showing the way in which the KRA of tissues, x (solid lines) tends to approach the falling curve for plasma KRA, y (broken line). Experimental plasma points taken from smoothed rat data after intraperitoneal injection (cf. Noonan et al., 1941) are indicated by circles. The values of K used in calculating values of x in the three curves were 5, 2, and 1. For equations see text. $y_o = 2.0$, $a = 0.8$ and $k = 0.383$.

Fig. 7. Experimental curves on an anesthetized rabbit to compare with the theoretical curves of figure 6. In this experiment the liver KRA does not approach the plasma KRA at a constant rate.

potassium which is removed in this way happens to contain a large fraction of the radioactive isotope, then the radioactive concentration will be high in the liver and if the radioactivity of the plasma happens to be falling rapidly by exchange with other tissues then the radioactivity of the liver potassium may be higher than that in the plasma and may remain so for some time.

In order to throw more light upon this peculiar power of certain organs for ingesting excess of potassium three experiments were tried on rabbits in which the radioactive potassium chloride solution was slowly infused

into the femoral vein by a motor driven syringe over a period of 10 minutes while samples of blood and liver were taken at intervals for analysis. The blood was taken from the carotid artery. Liver samples were removed in small pie-shaped pieces between ligatures with a minimum of bleeding. The animals were anesthetized with dial urethane. The figures from one of these experiments are given in table 5, and the values of radioactive/total K (KRA) for liver and plasma are given for the other two experiments in figures 7 and 8. These rabbits correspond respectively to nos. 7, 6 and 8 of table 2. The liver represented in figure 8 behaved very much like the theoretical curves of figure 6, as if a simple exchange by diffusion were the only process involved. In the other two experiments, particularly figure 7, the liver values were far in excess of the plasma values and showed no tendency to approach the plasma values during the course of the experiment. These experiments, therefore, represent the time of the peak in

TABLE 5

Absorption of radioactive potassium by rabbit liver

TIME	POTASSIUM		RA		KRA		RELATIVE KRA
	Plasma	Liver	Plasma	Liver	Plasma	Liver	
min.							
5	4.61	78.3	1.83	1.14	39.7	1.46	0.037
13	3.54	70.6	0.75	4.64	21.2	6.57	0.31
28	6.30	87.0	0.31	4.84	4.92	5.56	1.13
72	6.01	81*	0.24	4.3	3.98	5.31	1.33
133	5.72	70.3	0.16	3.65	2.85	5.19	1.82

Infusion of radioactive K lasted 11½ minutes. Dose = 1.9 m.eq. per kgm. Same as experiment 7 in table 2.

* Average.

the liver curve for relative KRA plotted in figure 4. Later in the experiment mixing would certainly become complete even in the liver and the liver and plasma curves would come to coincide fairly closely. Some permanent difference might be detected even at equilibrium on account of the greater atomic weight of the radioactive isotope (Fenn, Bale and Mullins, 1941) (Lasnitzki and Brewer, 1941).

The curves of figures 7 and 8 are presented as evidence that the liver, and presumably the other visceral organs to some degree, are able by some process to absorb potassium from the blood stream when the concentration of that element is for any reason excessively high in the plasma. In this process all the potassium isotopes probably behave alike or nearly so. Such an intake of potassium might occur by exchange with sodium but the analyses which we have made in cat livers before and after the injection of potassium lead us to suppose that the potassium is taken in with some

anion and water in isotonic solution (Fenn, 1939). Whatever may be the mechanism of this transfer, it appears to be partially a one-way process; at least the radioactive potassium goes into the liver more slowly than it comes out. A process of this sort is to be expected on the basis of the Boyle and Conway (1941) theory of the potassium equilibrium.

The rate with which potassium exchanges between plasma and tissues depends of course upon the blood supply of that tissue. Exchange is more rapid in active than in resting muscle (Noonan, Fenn and Haege, 1941a). Thus it is possible but not probable that the curves of figure 7 might be explained by assuming that the hepatic circulation was rapid while the radioactive potassium was being absorbed but thereafter, as a result of the trauma caused by the sampling, the circulation became so slow that the

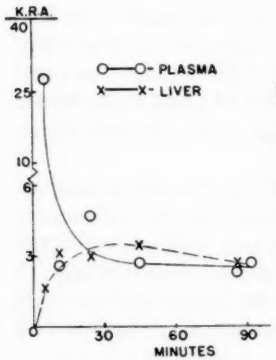


Fig. 8

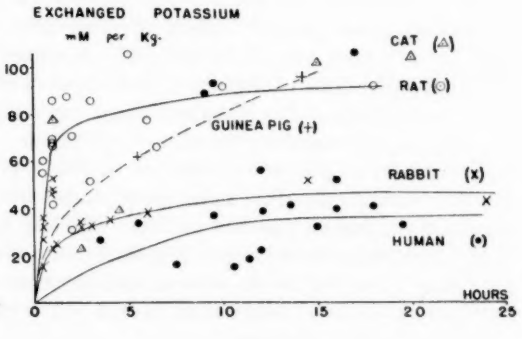


Fig. 9

Fig. 8. Similar to figure 7 but in this case the curves resemble the theoretical curves (cf. fig. 6) indicating that only a process of exchange of K^{42} for K^{39} is concerned.

Fig. 9. Amounts of body potassium exchanged with radioactive potassium at various times after injection. The total potassium in the body is about 80 mM per kgm.

liver could no longer maintain its equilibrium with the plasma and could not therefore dispose of its extra load of radioactive material. Since we were always careful to use a new lobe or a new part of the same lobe as far as possible from the location of the previous sample this does not appear very likely. Moreover, similar results are regularly obtained with unoperated and unanesthetized animals.

5. Total exchangeable potassium in man. On 10 different occasions in 5 different subjects we have made determinations of the total exchangeable potassium of the human body. The subject drank as large a sample of radioactive KCl as could conveniently be obtained and thereafter collected urine samples at intervals. The urine samples were usually evaporated and concentrated before analysis. It is assumed for purposes of the cal-

culation that the isotopic composition of the urine is the same as that of the plasma. If the urine is analyzed for both potassium and radioactivity it is possible to calculate the value of KRA according to the formula given and thus to estimate the total amount of potassium with which the ingested sample must have been mixed in order to give the proportion of radioactive to total potassium which was observed. Two determinations were also made in plasma and one determination in saliva. For this purpose 94 ml. of saliva and 38 ml. of plasma were collected and both were ashed in nitric acid and evaporated down to 4.5 ml. before analysis in order to increase the count. The KRA in both cases was 1.79 and the value in urine collected over a period beginning 2 hours before and ending 2 hours after the venipuncture had a value of 1.77. In this case therefore both saliva and urine served as good indices of the radioactivity of the plasma potassium. In another individual the KRA of the plasma was 5.5 and that of the corresponding urine was 6.5. Both of these latter values seem impossibly high for 11 hours after ingestion and are regarded with some suspicion. They indicate a total exchangeable potassium of only 18.1 and 15.5 mM per kgm. respectively. We obtained also 3 very high points in man which likewise appear erroneous for no evident reason.

The results of all our experiments of this type are plotted in figure 9, together with similar data taken from plasma analyses in our experiments on cats, rats, guinea pigs and rabbits. There is a very wide scatter in the points, especially those for man and the exact position of the curves is quite arbitrary. The results do seem to indicate that the rabbit and man mix their potassium less rapidly than the rat. The cat points are too few and divergent for any conclusion.

In the rats the total potassium of the body is about 70 mM per kgm. (Noonan, Fenn and Haege, 1941). In rabbits it is estimated as 79.2 in table 1 and we have made an independent estimate of 84 from our analyses of individual tissues and figures for the percentage of the total body weight due to each tissue. In order to be certain of this important figure we have dissolved a whole rabbit in nitric acid and have analyzed the digest for total K. In this way we found 82.9 mM per kgm. As already mentioned this is close to the potassium content found in cats by the same method (77.6). It is probable, therefore, that the total potassium in the human body is similar in magnitude. It may be concluded then that the cat, rat and guinea pig exchange all their body potassium within a period of perhaps 10 hours while man and the rabbit exchange only about half of it.

That there is a difference between species can be seen by comparing the rates with which the muscle potassium exchanges in rats and rabbits. The times for half exchange are about 1.5 hour in the rat and 7.5 hours in the rabbit. Since muscle accounts for 60 to 70 per cent of the total potassium of the body the relatively slow exchange of the muscle potassium of rabbits

explains satisfactorily the slow total exchange in that species. It seems possible that the slow exchange in rabbits and (sedentary) human subjects may be correlated with a sluggish muscle circulation. One of our subjects, however, tried to increase the rate of exchange of his potassium by several vigorous games of squash during the experiment but without noticeable success.

These low values in man and rabbit show that the ratio of $\frac{K^*}{K^+}$ (or KRA) is relatively too high in the plasma. In the case of the rabbit at least we have found that this high value in the plasma is not accompanied by an equally low value in the tissues. Thus as already mentioned the experiment of table 1 showed 80 per cent average exchange of body K by tissue analysis and only 59 per cent by plasma analysis. Thus more of the injected counts are exchanged in the tissue than would be predicted from the plasma analyses. This is not impossible and probably means that in some tissues the K^{42} is not completely mixed with the K^{39}. The plasma is equilibrated as well as possible with all tissues and therefore has a mean KRA and reflects in its own $\frac{K^{42}}{K^{39}}$ ratio the isotopic composition of the potassium in the tissues through which it passes. Since the K content of the plasma is never much increased as a result of the injection of K* it is evident that as soon as 10 minutes after injection practically all the K* (98–99 per cent of it) must be somewhere in the tissues and the average ratio of K^*/K^+ in the tissues cannot change. This does not mean however that mixing is complete. In general the viscera contain too much and the muscles and the skin too little. Indeed it may be that mixing is not always complete even within a single muscle. Muscles certainly take up some excess potassium by transport and must therefore acquire some K* in this way. There is no way to measure how rapidly such ingested K mixes with the previously contained K. If it is not completely mixed the circulating plasma may be equilibrated with the newly ingested K and may therefore have too high a K^{42}/K^{39} ratio. In other words, the KRA of plasma may be high because the plasma is still serving to convey K^{42} from places where it was "ingested" with an anion to places where it was simply exchanged for K^{39}. This probably happens in man and the rabbit to a greater extent than in the rat.

One other possible factor to explain the low exchange in man and rabbit may be a relative excess of the heavy potassium in urine as compared to plasma. We have discussed this possibility and presented our experimental evidence in another place (Fenn et al., 1941).

Special mention must be made of the work of Hevesy and Hahn (1941) on radioactive potassium in rabbits because they have reached the conclusion that less than half of the cellular potassium of the body can exchange with

plasma potassium whereas all our experience leads to the conviction that all of the potassium in the body will exchange in a few days and 90 per cent of it in 12 to 24 hours. Moreover, the experiments of Hevesy and Hahn lasted in general longer than ours. They find, however, only 33 per cent of the liver potassium exchanged in 64 hours. Indeed if all their figures were multiplied by a factor of 3 they would agree much better with our own.

It is evident that this discrepancy depends chiefly upon the value of the ratio of labelled to total K in the plasma. The results of Hevesy and Hahn agree with ours in showing that 95 per cent or more of the injected counts have escaped from the plasma a few minutes after an intravenous injection. The KRA of the plasma will thereafter serve as a measure of the average KRA of the potassium in all the tissues through which the blood passes. Examination of the figures of Hevesy and Hahn for radioactivity of the plasma indicates that they were regularly 2–3 times as high as ours. Table 2 shows 24 hour values of RA = 0.107 which is more typical of our results than the high value of 0.15 at 12 hours. In rats we have found only 0.08 in 18 hours and 0.094 in 6 hours and in cats 0.124 in 2.7 hours. In similar units we calculate from Hevesy and Hahn 0.2 for 24 hours as an average of 4 experiments and larger values for shorter times. There is evidently some fundamental difference in our results. Likewise they have found less penetration of erythrocytes than is indicated by our figures. On general principles moreover it seems very improbable that any appreciable fraction of the body potassium could escape exchange after several days.

The only explanation of these figures which we can think of is that Hevesy and Hahn used samples with a very slight contamination with sodium, say 0.01 per cent.

According to the "Tentative Table of Yields" reported by J. G. Hamilton (University of California) at the Conference on Applied Nuclear Physics held at Cambridge, Mass., 1940, the yields of Na^{24} and K^{42} for the 60 inch Berkeley cyclotron at 16 million volts are 10,000 and 50 microcuries per microampere hour. Thus 1 part of Na gives as many counts as 200 parts of K. Moreover it may be calculated that 1 cc. of plasma would contain about 0.006 per cent of the total body K as compared to 0.33 per cent of the total body Na. Hence in contributing to the final count in the plasma 1 part of sodium would be $200 \times \frac{0.33}{0.006} = 11{,}000$ times as effective as 1 part of K. Thus a contamination of Na of 0.01 per cent in the original KCl sample would make the final count just twice too high.[1] The calculated exchanges would then be twice too low. To avoid this error we have always added an equal weight of ordinary NaCl to the sample to

[1] Baker's analyzed C.P. potassium chloride is stated to contain 0.02 per cent of Na.

dilute any activated Na present, then precipitated out K as the perchlorate, filtered and reduced to KCl in the presence of MnO_2 by heat. We believe that this precaution accounts for the discrepancy between our results and those of Hevesy and Hahn.

In presenting our results we have made no attempt to calculate the exchange of cellular potassium after allowing for the fraction in each sample which must be assigned to the extracellular space. We recognize, however, the validity of this correction which was applied by Hevesy and Hahn although it does not significantly alter any of the conclusions.

SUMMARY

The distribution of radioactive potassium in the rabbit, cat and frog resembles in general the distribution in the rat as previously reported. The visceral organs take up the potassium more rapidly than skin, muscle, testis, brain and erythrocytes. During the first few hours after injection the ratio of K^{42} to K^{39} in the liver and other viscera is usually higher than in the plasma. Further analysis of this finding, both theoretical and experimental indicates that simple exchange of K^{42} for K^{39} by diffusion is not an adequate explanation. An excess of potassium in the plasma is probably quickly removed by the viscera together with an anion and is slowly released to the muscles.

In human subjects drinking solutions of radioactive potassium chloride determinations were made of the ratio of radioactivity to potassium in the urine. Calculation from these figures of the total exchangeable potassium of the body shows that man like the rabbit exchanges his body potassium less rapidly than the rat (50–60 per cent exchange in 12 hrs.).

The evidence indicates that in all animals studied practically all the potassium of the body exchanges with the injected radioactive material in the course of a few days.

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ELECTROLYTE CHANGES IN SUBMAXILLARY GLANDS DURING STIMULATION

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It has been reported previously (Wills and Fenn, 1938) that the potassium content of the submaxillary gland remained relatively constant during electrical stimulation. However, with pilocarpine injection the gland was found to lose potassium. The purpose of the present paper is to extend the original series of observations upon the effects of electrical and pilocarpine stimulations on the electrolyte content of the submaxillary gland to cover sodium, calcium, chlorine and phosphorus in addition to potassium. Further, some studies of the blood plasma contents of these elements during activity of the gland were carried out, and a few determinations were made of pH and lactic acid in blood and saliva.

EXPERIMENTAL. Cats or dogs anesthetised by intraperitoneal injection of Dial were used. The duct of one submaxillary gland was cannulated with a glass capillary.

If the gland was to be stimulated by electrical excitation of the chorda tympani, the chorda-lingual trunk ipsilateral to the prepared gland was cut as close as possible to the bulla. The peripheral section was arranged for stimulation through platinum electrodes from a thyratron with diphasic output. The saliva produced during the stimulation, which lasted for an average of 35 minutes, was collected in tared weighing bottles. At the end of the experiment the animal's aorta was sectioned. The stimulated submaxillary gland and the control gland from the opposite side of the neck were removed as rapidly as possible, carefully freed of extraneous tissues and blood, and placed in tared weighing bottles.

Two methods of stimulation with pilocarpine have been used. In one, the drug was injected into the general circulation through a femoral vein cannula. With this method the control gland had to be excised before commencement of stimulation. The second method of pilocarpine stimulation consisted of injection of the drug directly into the arterial circulation of the submaxillary gland from an arterial pump attached to the superior laryngeal artery, all other branches of the external carotid artery except the submaxillary division of the external maxillary artery being blocked

off. When the venous blood from the gland was diverted from the animal, as in the registration of blood flow, it was not necessary to remove the control gland until the end of this type of experiment.

In those experiments in which blood flow from the gland was recorded, the blood was prevented from coagulating by intravenous injection of 18 mgm. per kgm. body wt. of "Liquoid" (Hoffman-LaRoche) over a period of one-half hour. The venous blood was collected by placing a cannula in the external jugular vein after blocking off all tributaries except that from the submaxillary gland. Registration of blood and salivary flows by drops was achieved by use of an apparatus similar to that of Gesell (1929).

Arterial samples were taken from the deep femoral artery, and venous samples were taken directly from the outflow of the gland. Determination of the relative volumes of plasma and red cells in the blood samples was made after centrifugation at 3000 r.p.m. for thirty minutes in hematocrit tubes. Plasma samples were placed in tared weighing bottles.

All samples were dried to constant weight at 100°C. for determination of water content, and were then analysed. The samples were examined for chloride by the method of Van Slyke (1923) as modified by Wilson and Ball (1928) and Manery and Hastings (1939). Phosphorus was estimated by the method of Plimmer (1933), and calcium by that of Rappaport and Rappaport (1934). Phosphorus was removed before determination of sodium according to Overman and Garrett (1937). Sodium was determined by the procedure of Ball and Sadusk (1936), with reduction by cadmium amalgam and titration with ceric sulfate as advocated by Holmes and Kirk (1936). Potassium was determined by the method of Shohl and Bennett (1928) with the modifications recommended by Fenn *et al.* (1938). When the same sample was to be examined for more than one element, a scheme similar to that of Cullen and Wilkins (1933) was used. pH was determined with a glass electrode, and lactic acid was estimated by the method of Koenemann (1940).

This system of analysis yielded the following average recoveries when known amounts (within the expected experimental limits) of the various elements were added to samples of powdered rabbit muscle: Na 101.1 per cent, K 100.0 per cent, Ca 96.0 per cent, Cl 101.5 per cent and P 100.0 per cent. Standard deviations of the individual determinations from the mean were: Na $\pm$ 11.7 per cent, K $\pm$ 1.2 per cent, Ca $\pm$ 4.3 per cent, Cl $\pm$ 2.1 per cent and P $\pm$ 10.7 per cent. The sodium method in particular varied in reproducibility with the amount of element actually determined. When 0.2 mgm. of sodium was used, the standard deviation from the mean was $\pm$26.2 per cent. However, when 0.5 mgm. of sodium was used in the analysis, the standard deviation from the mean was only $\pm$1.7 per cent. Therefore, an attempt was made to have more than 0.5 mgm. of sodium in all samples analysed for that element.

The statistics used in this work, essentially those of Fisher (1936), are

$$s = \sqrt{\frac{\Sigma X^2}{n-1}} \qquad s_D = \sqrt{\frac{\Sigma X_1^2 + \Sigma X_2^2}{n_1 + n_2 - 2}}$$

$$t = \frac{\bar{X}_1 - \bar{X}_2}{s_D} \sqrt{\frac{n_1 n_2}{n_1 + n_2}}$$

s is the standard deviation of a single determination from the mean, s_D is the standard deviation of the differences of two means, X is the deviation of a single determination from the mean, $\bar{X}$, and n is the number of determinations in the series. The difference between two means was considered significant if the chance probability of its being zero was 5 per cent or less by the "t" test.

Table 1 shows averages of analyses of control and stimulated submaxillary glands of cats for water, sodium, potassium, calcium, chlorine and phosphorus. Stimulation was produced by either electrical excitation of the chorda tympani or by injection of pilocarpine. In the same table are average values for the rates of salivary secretion, for the salivary concentrations of the various electrolytes, and for the outputs in the saliva and intakes from the blood of the different elements. The last two quantities were calculated from the experimental measurements by the following formulae:

Output of element in m.eq. per 100 grams dry wt. of gland per hour ..... $= VS$

Intake of element in m.eq. per 100 grams dry wt. of gland per hour ..... $= VS + \frac{60(E_s - E_c)}{t}$

V is the milliliters of saliva secreted per gram dry weight of the gland per hour, S is the milli-equivalents of element per 100 ml. of saliva, E is the milli-equivalents of element per 100 grams dry weight of gland, t is the duration of secretion in minutes, and the subscripts s and c refer respectively to values for stimulated and control glands.

From table 1 it will be seen that, in cats, the composition of the saliva produced during stimulation of the submaxillary gland with pilocarpine was not significantly different from that of the saliva secreted during chorda excitation so far as potassium, calcium and phosphorus were concerned. However, the pilocarpine saliva had strikingly greater concentrations of sodium and chlorine. Asher (1908) had previously found that the NaCl content of the saliva was increased by intensive pilocarpine stimulation, and Vladesco and Nichita (1939) reported that intravenous injection of pilocarpine increased the chloride content of the saliva and slightly decreased that of the blood.

Such effects upon the composition of the saliva cannot be attributed to differences in rate of secretion with chorda and pilocarpine stimulations of the submaxillary gland. According to the work of Werther (1886), Gregerson and Ingalls (1931), Bienka and Szczepanski (1936), Brown and Klotz (1937), Bramkamp (1937) and Kesztyüs and Martin (1938a), both sodium and chlorine increase in concentration in the saliva with an increase in rate of secretion. On this basis the sodium and chlorine concentrations of pilocarpine saliva should have been somewhat lower than those of chorda saliva, for from table 1 it can be seen that the average rate of saliva production after pilocarpine injection was slightly below that with chorda

TABLE 1

Electrolytes of submaxillary glands of cats during stimulation (per 100 grams dry weight of gland or per 100 ml. of saliva)

ELEMENT DETERMINED	STIMULUS	NUMBER OF EXPERIMENTS	CONTROL GLAND				STIMULATED GLAND				SALIVA				TURNOVER BY GLAND			
			Water (ml.)		Element (m.eq.)		Water (ml.)		Element (m.eq.)		Flow (ml./ hour)		Element (m.eq.)		Intake (m.eq./ hour)		Output (m.eq./ hour)	
			$\bar{X}$†	s‡	X	s	$\bar{X}$	s	X	s	$\bar{X}$	s	$\bar{X}$	s	$\bar{X}$	s	$\bar{X}$	s
Sodium	Chorda	19	308	40	16.5	11.2	353	64	26.0	18.2	6210	3750	1.7	1.7	134.4	121.7	115.2	113.7
Sodium	Pilocarpine	12	312	32	12.3	3.0	372	56	30.7	20.5	4360	2000	5.5	3.5	296.7	207.0	262.7	217.7
Potassium	Chorda	36	314	20	35.6	3.8	358	49	35.8	4.3	4570	2560	0.91	0.34	39.2	21.5	39.0	21.0
Potassium	Pilocarpine	27	308	27	33.7	3.2	330	60	26.0	3.4	4410	1860	0.89	0.17	22.6	14.1	38.2	17.0
Calcium	Chorda	6	314	19	3.5	0.5	325	45	4.2	0.7	4530	1930	0.31	0.13	13.8	4.2	12.4	5.1
Calcium	Pilocarpine	6	320	29	3.9	1.2	362	36	3.0	0.7	3980	1600	0.22	0.06	7.1	4.4	8.8	4.2
Chlorine	Chorda	22	301	36	20.1	10.1	342	47	30.9	12.0	5440	3030	1.9	1.4	124.6	84.8	102.6	82.1
Chlorine	Pilocarpine	12	312	32	24.0	1.9	372	56	36.5	8.7	4360	2000	4.5	1.8	227.3	141.4	203.4	134.4
Phosphorus*	Chorda	6	314	19	23.6	5.7	325	45	24.8	6.5	4530	1930	0.14	0.08	8.0	3.9	5.6	3.3
Phosphorus	Pilocarpine	6	320	29	23.4	6.7	362	36	23.3	6.3	3980	1600	0.18	0.08	6.4	2.5	6.4	3.0

* All phosphorus figures in this paper are for total phosphorus. For calculation of the milliequivalents of element in a sample the phosphorus was assumed to have an effective valence of one.

† The average of the experimental values making up the sample.

‡ The standard deviation (±) of a simple determination. The standard deviation of the mean may be obtained by dividing this value by the square root of the number of individual determinations in the sample.

stimulation. It thus appears that pilocarpine had some specific effect in increasing the sodium and chlorine concentrations in the saliva.

Table 1 also shows that the only clearly significant changes in the composition of the submaxillary glands of cats during stimulation by electrical excitation of the chorda tympani were gains of water and chlorine. The probability that the gain in sodium was not real is only about 6 per cent, so that there may also have been an increase in the concentration of sodium in the gland. The calculated intakes from the blood were greater than the outputs in the saliva for all elements, but not significantly.

When pilocarpine stimulation was used, there were plainly significant gains of sodium and chlorine and a significant loss of potassium by the gland. The probability that there was no increase in the water content

of the gland is about 7 per cent. Potassium was the only element for which the intake was significantly different from the output. The average intake of potassium by the gland during pilocarpine stimulation was only 59.2 per cent of the average output in the saliva.

Table 2 contains data from a small series of dogs, the quantities measured being the same as those in table 1. It will be seen that here the saliva produced during pilocarpine stimulation had somewhat higher concentrations of calcium and phosphorus and a somewhat lower concentration of potassium than that produced during chorda stimulation. In these experiments the average rate of secretion with pilocarpine injection was only slightly greater than one-half that with chorda stimulation. It seems

TABLE 2

Electrolytes of submaxillary glands of dogs during stimulation (per 100 grams dry weight of gland or per 100 ml. of saliva)

ELEMENT DETERMINED	STIMULUS	CONTROL GLAND				STIMULATED GLAND				SALIVA				TURNOVER BY GLAND			
		Water (ml.)		Element (m.eq.)		Water (ml.)		Element (m.eq.)		Flow (ml./ hour)		Element (m.eq.)		Intake (m.eq./ hour)		Output (m.eq./ hour)	
		$\bar{X}$	s	$\bar{X}$	s	$\bar{X}$		$\bar{X}$	s	$\bar{X}$	s	$\bar{X}$	s	$\bar{X}$	s	$\bar{X}$	s
Sodium	Chorda	315	14	7.0	1.5	335	8	20.4	8.0	2990	1040	10.7	2.3	370	89	343	81
Sodium	Pilocarpine	306	15	12.2	4.3	328	25	18.3	6.0	1620	1080	10.7	5.3	188	126	176	120
Potassium	Chorda	315	14	43.7	2.6	335	8	44.0	2.3	2990	1040	1.7	0.1	57.1	18.9	56.5	15.4
Potassium	Pilocarpine	306	15	44.7	0.4	328	25	38.5	4.6	1620	1080	1.3	0.2	9.2	6.8	21.0	13.0
Calcium	Chorda	315	14	1.1	0.3	335	8	1.2	0.3	2990	1040	0.12	0.06	4.2	1.5	3.9	1.5
Calcium	Pilocarpine	306	15	1.2	0.4	328	25	1.3	0.2	1620	1080	0.26	0.06	4.2	2.3	4.1	2.6
Chlorine	Chorda	315	14	18.8	0.5	335	8	20.7	3.3	2990	1040	6.8	1.3	222	31	217	31
Chlorine	Pilocarpine	306	15	20.6	2.5	328	25	20.7	7.3	1620	1080	7.3	3.2	118	69	119	72
Phosphorus	Chorda	315	14	10.8	0.6	335	8	13.2	1.0	2990	1040	0.06	0.02	6.5	1.2	1.9	0.7
Phosphorus	Pilocarpine	306	15	9.8	1.0	328	25	11.2	0.9	1620	1080	0.20	0.05	6.3	4.0	3.6	3.1

Twelve animals are represented in this table, the submaxillary glands of six being stimulated by chorda excitation and those of the other six by pilocarpine injection. All samples were analysed for all the substances mentioned in the table. Therefore, each figure is the average of six analyses except those for salivary concentrations of the elements, intakes and outputs during chorda stimulation. Those figures are averages from five experiments, one saliva sample having been lost.

likely, therefore, that at similar rates of flow pilocarpine and chorda salivas of dogs would show the same chief differences of composition as do those of cats.

The average concentrations of sodium and chlorine in dog saliva were somewhat higher than those previously found (Gregersen and Ingalls, 1931; Kesztyüs and Martin, 1938a), and the average calcium concentration was only half that given by the above authors. No ready explanation for these differences can be found.

From table 2 it can be seen also that in dogs the submaxillary gland gained water, sodium and phosphorus during electrical stimulation. During pilocarpine stimulation also it gained phosphorus. The phos-

phorus results agree fairly well with those of Scoz and Lazari (1934), who found that most of the change was in the nucleic and fatty acid phosphate. With pilocarpine stimulation the probabilities that the increases in water and sodium concentrations were not real are respectively 10 per cent and 8 per cent. Phosphorus with chorda stimulation was the only element to have a significantly greater intake by the gland than output in the saliva. Potassium with pilocarpine stimulation had an average intake only 43.8 per cent of the output, but the probability that this difference was not real was 8 per cent. However, the potassium concentration in the gland did decrease significantly during pilocarpine stimulation. This indicates that there was actually less intake of potassium by the gland from the blood than was put out in the saliva during this type of stimulation.

Upon comparing the results of the experiments with cats and dogs it appears probable that in cats chlorine moved into the gland with sodium, while in dogs the anion accompanying sodium is not readily identifiable. It is of interest that in both dog and cat with pilocarpine stimulation potassium probably could be said to pass out of the gland in exchange for sodium, as it does in active muscle (Fenn and Cobb, 1936; Fenn *et al.*, 1938).

Table 3 contains some average values for arterial and venous plasma concentrations of potassium in cats, with averages of various other quantities from the same experiments. In addition, the results of a few individual experiments in which analyses for sodium, calcium, chlorine and phosphorus were also carried out are shown. Both electrical and pilocarpine stimulations were used. R represents the percent of plasma in whole blood, b is the rate of venous blood flow through the gland in milliliters per 100 grams dry weight of gland per hour, s is the secretion rate in milliliters per 100 grams dry weight of gland per hour, I is the intake of element by the gland in milli-equivalents per 100 grams dry weight of gland per hour, E represents the concentration of element in plasma in milli-equivalents per 100 ml., and the subscripts a and v indicate respectively arterial and venous values.

By comparing the figures for arterial and venous plasmas in this table it can be seen that in general the venous plasma was more concentrated with respect to sodium and chlorine than the arterial. These two elements are such a large part of the total electrolyte of the plasma that the venous blood always had a higher total electrolyte concentration in its plasma than the arterial blood, indicating that the submaxillary gland was doing osmotic work by abstracting from the blood more water than salts. This agrees with the known fact that the saliva is more dilute than the plasma. From the average potassium figures it can be seen that with chorda stimulation the plasma in passing through the gland became less concentrated with respect to potassium, while after pilocarpine injection

it became more concentrated. This finding can probably be explained by the smaller intake of potassium from the blood by the gland during pilocarpine stimulation, and by the previous demonstration that for a given saliva flow the blood flow through the gland with pilocarpine stimulation was considerably smaller than with chorda excitation (Wills, 1941).

It should be possible to calculate the venous plasma concentration of an element from the other quantities in table 3 by the following expression:

$$E_{V_1} = \frac{E_a R_a(b + s) - 10{,}000I}{R_V b}$$

The figures in the last column of the table were obtained in this way. When the calculated venous plasma concentrations are compared with the actual ones it is found that the agreement is not very good. This is not surprising in view of all the chances for error in the data, and it is held to be distinctly encouraging that for 63 per cent of the attempts it was possible to predict by this expression the direction of the change in plasma concentration.

By comparing the figures for arterial and venous blood plasma concentrations of the various elements in table 3 with those for saliva concentrations in table 1, it can be seen that the saliva concentration of none of the elements studied in this paper was intermediate to the arterial and venous plasma concentrations of the same element. The concentration of potassium in the saliva was greater than that in either arterial or venous plasma, and the saliva concentrations of the other elements were lower than the respective concentrations in plasma. It thus appears that the submaxillary gland in some way passes into the saliva from the blood proportionately more potassium and water than the other substances studied. These findings agree with those of deBeer and Wilson (1932) for parotid saliva of dogs except in the case of calcium. These authors found that the calcium content of the parotid saliva obtained by pilocarpine stimulation was greater than that of blood serum. Kesztyüs and Martin (1938b), however, reported that in the dog the saliva elicited by chorda stimulation had a calcium concentration between those of arterial and venous bloods. It has already been pointed out that the values for saliva calcium found in the present work are lower than those previously reported although the blood plasma figures are satisfactory. The location of the error awaits further work.

Table 4 contains the average results of some experiments in which arterial, venous and saliva samples were collected under oil for determination of pH. The samples were then equilibrated at room temperature with a mixture of 5 per cent carbon dioxide and 95 per cent oxygen, and the pH redetermined. The latter procedure gives a measure of changes in the alkali reserve. The figures show that the saliva was always more basic

than the blood, agreeing with the work of Brassfield (1936). The fact that pilocarpine saliva became more basic after equilibration with the 5 per cent CO_2-95 per cent O_2 mixture while chorda saliva became more acid must mean that with pilocarpine stimulation the CO_2 tension in the saliva

TABLE 3

Electrolytes in blood passing through submaxillary glands of cats during stimulation

See text for definition of column headings

EXPERIMENT NUMBER	STIMULUS	ELEMENT	R_A	R_V	b	s	I	E_A	E_V	
									Obs.	Calc.
28	Chorda	Sodium	49.6	53.8	8200	2939	26.3	15.0	21.6	18.2
		Potassium					57.0	0.36	0.39	0.45
		Calcium					10.4	0.62	0.38	0.54
		Chlorine					92.6	12.1	13.7	13.0
		Phosphorus					9.1	0.48	0.51	0.39
30	Chorda	Sodium	46.7	44.4	12,740	1407	9.0	14.7	19.1	17.0
		Potassium					20.2	0.35	0.44	0.35
		Calcium					8.6	0.69	0.42	0.65
		Chlorine					42.9	11.4	14.6	12.5
		Phosphorus					8.3	0.29	0.39	0.19
31	Chorda	Sodium	65.2	65.8	39,670	3881	40.5	8.1	16.1	8.7
		Potassium					48.1	0.63	0.68	0.50
		Calcium					12.9	0.91	0.44	0.94
		Chlorine					76.4	12.9	12.2	13.7
		Phosphorus					1.5	0.40	0.37	0.43
Average of 12.....		Potassium	64.5	63.6	17,865	2641	40.4	0.56	0.50	0.46
29	Pilocarpine	Sodium	58.1	51.6	9945	2988	64.5	16.2	17.7	22.4
		Potassium					2.8	0.57	0.74	0.78
		Calcium					3.7	0.60	0.53	0.81
		Chlorine					92.5	12.2	14.2	16.1
		Phosphorus					8.0	0.33	0.48	0.33
32	Pilocarpine	Sodium	53.9	46.0	19,460	5296	332.0	10.0	15.5	11.2
		Potassium					26.8	0.34	0.37	0.21
		Calcium					7.2	0.51	0.45	0.68
		Chlorine					133.2	12.5	14.4	17.1
		Phosphorus					4.5	0.25	0.34	0.32
Average of 20.....		Potassium	58.4	51.9	22,535	3740	29.8	0.53	0.62	0.55

was greater than that corresponding to 5 per cent CO_2 while with chorda excitation it was less. The higher CO_2 tension in pilocarpine saliva as compared with chorda saliva would explain the greater secretion of bicarbonate in the former type of saliva found by McClanahan and Amber-

son (1935). The values of blood pH after equilibration support the suggestion of these authors that the large elimination of bicarbonate in the saliva during pilocarpine administration might lead to a considerable diminution in the alkali reserve of the blood.

Table 5 contains some figures for the lactic acid concentrations of arterial and venous plasmas and of saliva. The figures show that the blood lactic acid was definitely increased during activity of the submaxillary gland following either type of stimulation. It is interesting that there was more than three times as much lactic acid in pilocarpine saliva as in chorda saliva, although there was no marked difference in the rates of secretion with the two types of stimuli. This finding is in agreement with the work of Vladesco and Nichita (1936) in which it was found that in the

TABLE 4

pH of blood and submaxillary saliva of cats

STIMULUS	NUMBER OF EXPERIMENTS	ARTERIAL BLOOD		VENOUS BLOOD		SALIVA	
		As drawn	5 per cent CO_2	As drawn	5 per cent CO_2	As drawn	5 per cent CO_2
Chorda	5	7.34	7.23	7.31	7.21	7.95	7.43
Pilocarpine	3	7.41	7.21	7.32	7.12	7.56	7.63

TABLE 5

Lactic acid content of blood and submaxillary saliva of cats (mgm. per cent)

STIMULUS	NUMBER OF EXPERIMENTS	ARTERIAL BLOOD	VENOUS BLOOD	SALIVA
Resting	1	7.40	8.75	
Chorda	2	6.62	16.12	3.42
Pilocarpine	2	6.95	14.60	12.71

dog pilocarpine saliva contained on the average 3.6 times as much lactic acid as chorda saliva. However, these authors also found that the venous blood from the gland after pilocarpine injection contained almost twice as much lactic acid as did that collected during chorda stimulation. The lactic acid concentrations of venous bloods with pilocarpine and chorda stimulations in our cats were essentially the same, indicating perhaps a somewhat different effect of pilocarpine on the metabolism of the submaxillary gland in dog and cat.

Such increased lactic acid excretion in the saliva during pilocarpine injection may aid in explaining the greater output of base with this type of stimulation. From table 1 it can be seen that the increased output of sodium in the saliva with pilocarpine stimulation as compared with chorda excitation was not completely balanced by the increase in chlorine output. This discrepancy amounted to 1.2 m.eq. for 100 ml. of saliva. It can be

calculated that, as a rough approximation, the greater bicarbonate secretion in pilocarpine saliva can account for 1.1 m.eq. of this excess sodium, while the relatively large lactic acid excretion in the saliva during pilocarpine administration can account for about 0.1 m.eq.

The findings that pilocarpine saliva contained several times as much lactic acid as chorda saliva and had a greater CO_2 tension are perhaps correlated with the previous demonstration that at equal rates of secretion the submaxillary gland stimulated with pilocarpine had less blood circulating through it than the gland stimulated by chorda excitation (Wills, 1941). Such relative ischemia of the gland stimulated by pilocarpine injection would result in less removal of lactic acid and CO_2 from the active tissue by the blood, and this in turn would mean that the saliva during its production would be in equilibrium with higher concentrations of these two substances. Hence, the CO_2 tension and lactic acid concentration in pilocarpine saliva should be greater than those of chorda saliva.

SUMMARY

During stimulation by pilocarpine injection the submaxillary glands of both dogs and cats were found (probably) to gain water and sodium while losing potassium. During stimulation by chorda excitation the gland gained water and sodium without losing an important amount of any of the other elements determined.

The submaxillary saliva secreted in response to injection of pilocarpine differed from chorda saliva by having higher concentrations of sodium and chlorine. The pilocarpine saliva of cats also contained about 3.7 times as much lactic acid as chorda saliva, and had a greater CO_2 tension.

The submaxillary glands of cats did osmotic work during secretion, concentrating the plasma with respect to the sum of the electrolytes studied. The gland apparently abstracted from the blood and passed into the saliva proportionately more water and potassium than other electrolytes.

None of these findings explains the unique loss of potassium by the submaxillary gland after pilocarpine injection.

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THE EFFECT OF ADRENAL CORTICAL HORMONE ON THE SYNTHESIS OF CARBOHYDRATE IN LIVER SLICES[1,2]

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The rôle of the adrenal cortical hormone in regulating carbohydrate metabolism has been subject to much investigation during the past decade (1, 1a). It has already been shown (1a, 2, 3, 4) that adrenalectomized animals have a decreased ability to form carbohydrate from non-carbohydrate precursors and that patients with Addison's disease (5) and adrenalectomized animals (4) utilize available carbohydrate more rapidly than normal controls. This increase in carbohydrate utilization can be depressed by treatment with aqueous adrenal cortical extract, corticosterone or 17-hydroxy-11-dehydro-corticosterone (4, 5, 6).

The ability of liver slices of normal rats to form measurable quantities of carbohydrate has been reported by Gemmill (7). More recently Holmes and Lehmann (8) reported that they observed no difference in the formation of total carbohydrate from lactate in liver slices of normal and adrenalectomized rats. It has been shown in other studies (9, 10, 11) that the adrenal cortical hormone increases the oxygen consumption of liver and kidney slices with or without the addition of substrate.

It seems reasonable to infer from data obtained on intact animals that the adrenal cortical carbohydrate-regulating factor exerts a pronounced effect on the liver. As this assumption has not been proved, an attempt has been made in this study to increase our understanding of the action of the adrenal cortex by investigating the effect of adrenal cortical hormone on the formation of total carbohydrate in liver slices.

METHODS. Rat liver slices were studied in vitro by means of the Warburg apparatus. Tissue was obtained from 200 to 250 gram rats of the Sprague-Dawley strain maintained on a diet of Purina dog chow. The rats were grouped as follows: *a*. Normal rats. *b*. Adrenalectomized rats.

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c. Normal rats treated with adrenal cortical extract. *d*. Adrenalectomized rats treated with adrenal cortical extract. Adrenalectomized rats were maintained in good condition by the addition of 1 per cent sodium chloride to the drinking water. The completeness of adrenalectomy was confirmed by post-mortem examination and by finding a lower-than-normal total carbohydrate content of the liver following a 24-hour fast. The treated animals (groups *c* and *d*) were given a single injection of 5 ml. of adrenal cortical extract (Wilson[4]) intraperitoneally one hour before the animals were sacrificed.

Following a 24-hour fast the animals were stunned by a sharp blow on the head, decapitated and the liver removed and placed in cold Ringer's solution. Slices of liver less than 0.3 mm. in thickness were cut by hand, weighed on a torsion balance and placed directly into Warburg vessels, each containing 2 ml. of the appropriate media. Approximately 80 mgm. of tissue were used in each vessel. All experiments were carried out at a temperature of 37.4°C. using a gas phase of 100 per cent oxygen.

The following substrates were used: 1. Phosphate-buffered Ringer's solution. 2. d-Lactate (Pfanstiehl) 0.4 per cent. 3. Pyruvate (Eastman) 0.4 per cent. 4. d-l-Alanine (Eastman) 0.4 per cent. 5. d-Glutamate (Eastman) 0.4 per cent. All substrates were dissolved in phosphate-buffered Ringer's solution and the pH adjusted to 7.4 with sodium hydroxide.

The total carbohydrate content of the liver slices in some vessels was determined ten minutes after the vessels were placed in the Warburg apparatus. This was designated as initial value. Final total carbohydrate determinations were made on the slices in the remaining vessels after $2\frac{1}{4}$ hours. The oxygen consumption was measured at $\frac{1}{2}$ hour intervals during the last 2 hours of this period. Carbon dioxide was removed by means of 0.1 ml. of 20 per cent potassium hydroxide placed on filter paper in the center well of the Warburg vessel.

To determine total carbohydrate, the slices and media were transferred into a 40 ml. centrifuge tube containing 1 ml. of 4 N sulphuric acid, the Warburg flask being rinsed with 1 ml. of distilled water. Thus the resulting concentration of sulphuric acid in the centrifuge tube was 1 N. This mixture was then hydrolyzed for three hours in a boiling water bath. After neutralization with sodium hydroxide, protein was precipitated by the zinc sulfate–sodium hydroxide method of Somogyi (12)[5]. Glucose was then determined by the Schaffer-Hartmann method (14). Values were expressed as milligrams of carbohydrate per 100 mgm. of tissue (wet weight).

[4] The adrenal cortical extract was supplied through the courtesy of Dr. David Klein, Wilson Laboratories, Chicago, Ill.

[5] Mirsky and Somogyi (13) have recently pointed out that the use of the zinc-sodium hydroxide method of deproteinization gives a low absolute sugar value.

OBSERVATIONS. I. *Formation of carbohydrate by liver slices.* (a) *Ringer's solution without added substrate.* Liver slices of all four groups of rats formed a constant but small quantity of total carbohydrate in a medium of Ringer's solution without added substrate (table 1, fig. 1). Neither adrenalectomy nor treatment with adrenal cortical extract affected the rate of formation of total carbohydrate in this medium.

(b) *Ringer's solution + d-l-alanine 0.4 per cent.* The amount of total carbohydrate formed from d-l-alanine was approximately the same as the endogenous formation in a medium of plain Ringer's solution (table 2). A limited number of experiments were carried out with substrates of d-alanine

TABLE 1

Effect of adrenal cortical hormone on the synthesis of total carbohydrate in rat liver slices
Media: Ringer's solution

SOURCE OF LIVER SLICES	NUMBER OF SLICES	INCREASE IN TOTAL CARBOHYDRATE (MEAN)**	DIFFERENCE BETWEEN MEANS	"P"‡
Normal untreated	15	0.07 ±0.035†		
Adrenalectomized untreated	10	0.06 ±0.033	−0.01	0.50
Normal untreated	15	0.07 ±0.035		
Normal treated*	10	0.11 ±0.075	+0.04	0.10
Adrenalectomized untreated	10	0.06 ±0.033		
Adrenalectomized treated*	9	0.04 ±0.036	−0.02	0.20

* Five cubic centimeters of Wilson adrenal cortical extract (see Methods).

** Mgm. per 100 mgm. tissue.

† Standard deviation, $\sqrt{\frac{\Sigma\Delta^2}{n}}$

‡ "P" derived by use of Fisher's table of "t" (15).

$$\text{"t"} = \frac{\text{Difference between the means}}{\text{Standard error of the difference between means}}$$

In comparing two groups the difference between the means is significant if P is <0.05.

§ Cubic millimeters of oxygen per hour per milligram of tissue (wet weight) Tables 8 and 9.

and l-alanine and it was found that neither of these substances increased the rate of total carbohydrate formation in liver slices of normal rats. These observations are at variance with those reported by Cross and Holmes (16) and Stadie et al. (17). Furthermore, neither adrenalectomy nor treatment with adrenal cortical extract altered significantly the amount of carbohydrate formed from d-l-alanine by rat liver slices (table 3, fig. 1).

(c) *Ringer's solution + d-glutamate 0.4 per cent.* When d-glutamate was used as a substrate, liver slices of all four groups of animals formed a greater amount of carbohydrate than in a medium of plain Ringer's solu-

tion (table 2). This increase was of the same magnitude in three of the four groups, i.e., normal, adrenalectomized and adrenalectomized treated with adrenal cortical extract (table 4, fig. 1). The increase in total carbohydrate formation in the normal rats treated with hormone, however, was only 0.21 as compared to an increase of 0.30 in the untreated controls.

(d) *Ringer's solution + pyruvate 0.4 per cent.* There was a marked increase in total carbohydrate formation in the liver slices of all groups in a medium which contained pyruvate (table 2). However, the carbohydrate

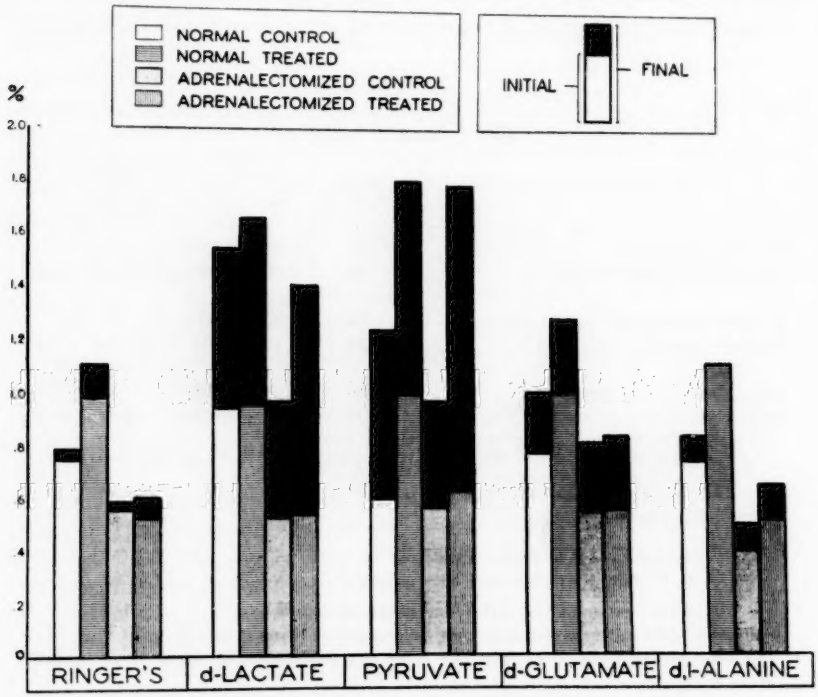

Fig. 1. Effect of adrenal cortical hormone on synthesis of total carbohydrate by rat liver slices. Total CHO content of liver slice.

increment was only 0.44 in liver slices from adrenalectomized rats as compared to 0.64 in liver slices of normal rats (table 5, fig. 1).

The total carbohydrate formation in the presence of pyruvate in normal rats treated with adrenal cortical extract was increased to 0.87 as compared with a normal increment of 0.64 (table 5, fig. 1). The liver slices of adrenalectomized rats treated with adrenal cortical extract showed the most marked carbohydrate formation from pyruvate, the increase being 1.18 as compared to 0.44 in the untreated adrenalectomized group (table 5, fig. 1).

(e) *Ringer's solution + d-lactate 0.4 per cent.* Carbohydrate formation was markedly increased in all groups by the addition of d-lactate to the medium (table 2). With this substrate the normal and adrenalectomized groups did not differ significantly (table 6, fig. 1) but a further increase in

TABLE 2

Effect of substrate on the synthesis of total carbohydrate in rat liver slices

SUBSTRATE	NORMAL				ADRENALECTOMIZED			
	Number of slices	Increase in total carbohydrate (mean)	Difference between means	"P"‡	Number of slices	Increase in total carbohydrate (mean)	Difference between means	"P"‡
Ringer's solution	15	0.07 ±0.035†			10	0.06 ±0.035†		
d,l-Alanine	12	0.07 ±0.050	0		10	0.10 ±0.048	+0.04	0.04
Ringer's solution	15	0.07 ±0.035			10	0.06 ±0.033		
d-Glutamate	10	0.30 ±0.085	+0.23	<0.01	9	0.28 ±0.093	+0.22	<0.01
Ringer's solution	15	0.07 ±0.035			10	0.06 ±0.033		
Pyruvate	9	0.64 ±0.173	+0.57	<0.01	10	0.44 ±0.118	+0.38	<0.01
Ringer's solution	15	0.07 ±0.035			10	0.06 ±0.033		
d-Lactate	20	0.54 ±0.141	+0.47	<0.01	14	0.56 ±0.230	+0.50	<0.01
	NORMAL TREATED*				ADRENALECTOMIZED TREATED*			
	Number of slices	Increase in total carbohydrate (mean)	Difference between means	"P"‡	Number of slices	Increase in total carbohydrate (mean)	Difference between means	"P"‡
Ringer's solution	10	0.11 ±0.075†			9	0.04 ±0.036†		
d,l-Alanine	9	0.08 ±0.052	−0.03	0.30	10	0.12 ±0.053	+0.08	<0.01
Ringer's solution	10	0.11 ±0.075			9	0.04 ±0.036		
d-Glutamate	10	0.21 ±0.068	+0.10	<0.01	10	0.29 ±0.051	+0.25	<0.01
Ringer's solution	10	0.11 ±0.075			9	0.04 ±0.036		
Pyruvate	10	0.87 ±0.243	+0.76	<0.01	11	1.18 ±0.150	+1.14	<0.01
Ringer's solution	10	0.11 ±0.075			9	0.04 ±0.036		
d-Lactate	8	0.89 ±0.263	+0.78	<0.01	6	0.85 ±0.135	+0.81	<0.01

See table 1 for footnotes.

carbohydrate synthesis (from 0.54 to 0.89) was noted in the normal group treated with adrenal cortical extract. The adrenalectomized treated group also showed a marked rise in carbohydrate formation increasing from 0.56 to 0.85.

II. *The total carbohydrate content of liver following a 24-hour fast.* Fol-

lowing a 24-hour fast, the total carbohydrate content of liver of adrenalectomized animals was much lower than that of the normal group (table 7). Adrenal cortical extract injected one hour prior to removal of the liver raised the average total carbohydrate content of the liver of normal rats from 0.75 to 0.98 but did not alter the carbohydrate content of the liver of adrenalectomized rats. These observations indicate that the action of the hormone takes place rapidly, at least in normal animals. The failure of hormone treatment to increase the carbohydrate content of the liver of adrenalectomized animals within 1 hour appeared to be due to insufficient time for the hormone to act in the case of these animals and possibly to a deficiency of carbohydrate precursors rather than to an inability of the tissue to respond, since liver *slices* of adrenalectomized,

TABLE 3

Effect of adrenal cortical hormone on the synthesis of total carbohydrate in rat liver slices

Media: Ringer's solution + *d-l-Alanine* 0.4 per cent

SOURCE OF LIVER SLICES	NUMBER OF SLICES	INCREASE IN TOTAL CARBOHYDRATE (MEAN)	DIFFERENCE BETWEEN MEANS	"P"‡
Normal untreated	12	0.07 ±0.050†		
Adrenalectomized untreated	10	0.10 ±0.048	+0.03	0.20
Normal untreated	12	0.07 ±0.050		
Normal treated*	9	0.08 ±0.052	+0.01	0.70
Adrenalectomized untreated	10	0.10 ±0.048		
Adrenalectomized treated*	10	0.12 ±0.053	+0.02	0.70

See table 1 for footnotes.

TABLE 4

Effect of adrenal cortical hormone on the synthesis of total carbohydrate in rat liver slices

Media: Ringer's solution + *d-glutamate* 0.4 per cent

SOURCE OF LIVER SLICES	NUMBER OF SLICES	INCREASE IN TOTAL CARBOHYDRATE (MEAN)	DIFFERENCE BETWEEN MEANS	"P"‡
Normal untreated	10	0.30 ±0.085†		
Adrenalectomized untreated	9	0.28 ±0.093	−0.02	0.60
Normal untreated	10	0.30 ±0.085		
Normal treated*	10	0.21 ±0.068	−0.09	0.02
Adrenalectomized untreated	9	0.28 ±0.093		
Adrenalectomized treated*	10	0.29 ±0.051	+0.01	0.80

See table 1 for footnotes.

extract-treated rats formed carbohydrate from non-carbohydrate precursors as well as, if not better than, those of normal rats.

III. *Oxygen consumption of liver slices following a 24-hour fast.* An increase in oxygen uptake was noted in all four groups of animals upon the addition of d-l-alanine, d-glutamate or pyruvate to the media (table 8). Oxygen uptake was also increased upon the addition of d-lactate to the

TABLE 5

Effect of adrenal cortical hormone on the synthesis of total carbohydrate in rat liver slices

Media: Ringer's solution + *Pyruvate* 0.4 per cent

SOURCE OF LIVER SLICES	NUMBER OF SLICES	INCREASE IN TOTAL CARBOHYDRATE (MEAN)	DIFFERENCE BETWEEN MEANS	"P"‡
Normal untreated	9	0.64 ±0.173†		
Adrenalectomized untreated	10	0.44 ±0.118	−0.20	<0.01
Normal untreated	9	0.64 ±0.173		
Normal treated*	9	0.87 ±0.243	+0.23	0.03
Adrenalectomized untreated	10	0.44 ±0.118		
Adrenalectomized treated*	11	1.18 ±0.150	+0.74	<0.01

See table 1 for footnotes.

TABLE 6

Effect of adrenal cortical hormone on the synthesis of total carbohydrate in rat liver slices

Media: Ringer's solution + *d-lactate* 0.4 per cent

SOURCE OF LIVER SLICES	NUMBER OF SLICES	INCREASE IN TOTAL CARBOHYDRATE (MEAN)	DIFFERENCE BETWEEN MEANS	"P"‡
Normal untreated	20	0.54 ±0.141†		
Adrenalectomized untreated	14	0.56 ±0.230	+0.02	0.80
Normal untreated	20	0.54 ±0.141		
Normal treated*	8	0.89 ±0.263	+0.35	<0.01
Adrenalectomized untreated	19	0.56 ±0.230		
Adrenalectomized treated*	6	0.85 ±0.135	+0.29	<0.01

See table 1 for footnotes.

media in the two groups in which it was studied (table 8), i.e., "normal untreated" and "normal treated."

A study of table 9 indicates that in these experiments adrenal cortical hormone had *no consistent effect* on the *rate* of *oxygen uptake.* In plain Ringer's the rate of oxygen uptake was increased in the adrenalectomized, untreated group as well as in the adrenalectomized and normal groups

treated with extract when compared to the normal, untreated group. No significant difference was observed between the increased oxygen uptake of adrenalectomized untreated and adrenalectomized treated groups. When d-l-alanine was added as a substrate, although the rate of oxygen uptake was increased in all of the groups, no significant difference was noted among the several groups. In the presence of d-glutamate, although the rate of oxygen uptake was again increased in all of the groups, the values for the adrenalectomized untreated and treated, as well as the normal untreated exceeded that of the normal group treated with extract. The increase in oxygen uptake of the adrenalectomized, treated group was significantly greater than that of the adrenalectomized, untreated and control, untreated groups. With pyruvate as a substrate, although the oxygen uptake of all four groups was greatly increased, there was no significant difference among the groups except in the case of the adrenalectomized, treated

TABLE 7

Effect of adrenal cortical hormone on total carbohydrate content of the liver of rats fasted 24 hours

SOURCE OF LIVER	NUMBER OF DETERMINATIONS	TOTAL CARBOHYDRATE CONTENT OF LIVER (MEAN)	DIFFERENCE BETWEEN MEANS	"P"‡
Normal untreated	14	0.75 ±0.159†		
Adrenalectomized untreated	10	0.55 ±0.054	−0.20	<0.01
Normal untreated	14	0.75 ±0.159		
Normal treated*	11	0.98 ±0.251	+0.23	0.01
Adrenalectomized untreated	10	0.55 ±0.054		
Adrenalectomized treated*	9	0.52 ±0.078	−0.03	0.30

See table 1 for footnotes.

group which was greatly increased when compared to the untreated, adrenalectomized group. No difference was observed in the increase in rate of oxygen utilization between the normal, untreated group and the normal, extract-treated group when d-lactate was used as a substrate.

Discussion. The effect of adrenal cortical hormone in increasing the rate of formation of carbohydrate from pyruvate or lactate in liver slices is consistent with the findings in intact animals previously reported (4) and provides additional evidence that the adrenal cortical hormone facilitates this reaction by acting on the liver.

Although treatment with adrenal cortical extract is followed by a marked increase in carbohydrate synthesis from lactic acid, in our experiments as in those of Holmes and Lehmann (8) liver slices of untreated adrenalectomized rats formed carbohydrate at approximately the same rate as liver slices from normal animals.

Recent studies (18, 19, 20) and our own unpublished data indicate that carbohydrate is readily synthesized from non-carbohydrate sources by *kidney slices*, although Mirsky et al. (13) in a recent paper have subscribed to the generally accepted theory "that the liver is the only source of endogenous glucose." Although it is apparent that the liver is *not* the only

TABLE 8

Effect of substrate on the oxygen consumption of rat liver slices

SUBSTRATE	NORMAL				ADRENALECTOMIZED			
	Number of slices	Oxygen consumption (mean)§	Difference between means	"P"‡	Number of slices	Oxygen consumption (mean)§	Difference between means	"P"‡
Ringer's solution	14	1.70 ±0.104†			10	1.88 ±0.135†		
d,l-Alanine	10	2.24 ±0.196	+0.54	<0.01	10	2.29 ±0.052	+0.41	<0.01
Ringer's solution	14	1.70 ±0.104			10	1.88 ±0.135		
d-Glutamate	12	2.46 ±0.223	+0.76	<0.01	10	2.55 ±0.161	+0.67	<0.01
Ringer's solution	14	1.70 ±0.104			10	1.88 ±0.135		
Pyruvate	10	3.43 ±0.614	+1.73	<0.01	10	3.06 ±0.503	+1.18	<0.01
Ringer's solution	14	1.70 ±0.104						
d-Lactate	11	3.58 ±0.564	+1.88	<0.01				
	NORMAL TREATED*				ADRENALECTOMIZED TREATED*			
	Number of slices	Oxygen consumption (mean)§	Difference between means	"P"‡	Number of slices	Oxygen consumption (mean)§	Difference between means	"PP"‡
Ringer's solution	10	1.86 ±0.237†			9	1.88 ±0.142†		
d,l-Alanine	10	2.29 ±0.287	+0.43	<0.01	10	2.28 ±0.307	+0.40	<0.01
Ringer's solution	10	1.86 ±0.237			9	1.88 ±0.142		
d-Glutamate	10	2.24 ±0.205	+0.38	<0.01	10	2.77 ±0.173	+0.89	<0.01
Ringer's solution	10	1.86 ±0.237			9	1.88 ±0.142		
Pyruvate	12	3.26 ±0.549	+1.40	<0.01	10	3.53 ±0.208	+1.65	<0.01
Ringer's solution	10	1.86 ±0.237						
d-Lactate	10	3.37 ±0.405	+1.51	<0.01				

See table 1 for footnotes.

source of endogenous glucose, it may well be that the liver is the only *depot* of readily available carbohydrate since muscle glycogen is not readily mobilized and since the glycogen content of normal kidney is insignificant.

The concept of multiple sites of possible endogenous carbohydrate formation introduces several considerations regarding the manner in which

the adrenal cortical hormone effects the conversion of amino acids to carbohydrate. Apparently in the liver slice, adrenal cortical hormone

TABLE 9

Effect of adrenal cortical hormone on the oxygen consumption of rat liver slices

MEDIA	SOURCE OF LIVER SLICES	NUMBER OF SLICES	OXYGEN CONSUMPTION (MEAN)§	DIFFERENCE BETWEEN MEANS	"P"‡
Ringer's solution	Normal untreated	14	1.70 ±0.104†		
	Adrenalectomized untreated	10	1.88 ±0.135	+0.18	<0.01
	Normal untreated	14	1.70 ±0.104		
	Normal treated*	10	1.86 ±0.237	+0.16	0.05
	Adrenalectomized untreated	10	1.88 ±0.135		
	Adrenalectomized treated*	9	1.88 ±0.142	0	
d,l-Alanine	Normal untreated	10	2.24 ±0.196		
	Adrenalectomized untreated	10	2.29 ±0.052	+0.05	0.60
	Normal untreated	10	2.24 ±0.196		
	Normal treated*	10	2.29 ±0.287	+0.05	0.60
	Adrenalectomized untreated	10	2.29 ±0.052		
	Adrenalectomized treated*	10	2.28 ±0.307	−0.01	0.90
d-Glutamate	Normal untreated	12	2.46 ±0.223		
	Adrenalectomized untreated	10	2.55 ±0.161	+0.09	0.30
	Normal untreated	12	2.46 ±0.223		
	Normal treated*	10	2.24 ±0.205	−0.22	0.02
	Adrenalectomized untreated	10	2.55 ±0.161		
	Adrenalectomized treated*	10	2.77 ±0.173	+0.22	<0.01
Pyruvate	Normal untreated	10	3.43 ±0.614		
	Adrenalectomized untreated	10	3.06 ±0.503	−0.37	0.20
	Normal untreated	10	3.43 ±0.614		
	Normal treated*	12	3.26 ±0.549	−0.17	0.50
	Adrenalectomized untreated	10	3.06 ±0.503		
	Adrenalectomized treated*	10	3.53 ±0.208	+0.47	0.01
d-Lactate	Normal untreated	11	3.58 ±0.564		
	Normal treated*	10	3.37 ±0.405	−0.21	0.30

See table 1 for footnotes.

failed to increase significantly the rate of formation of pyruvic acid from alanine or glutamate (table 3) for if it had done so the rate of carbohydrate

synthesis would probably have been increased, as the experiments in which pyruvate itself was added as a substrate indicate (table 5). It is possible that the acceleration of the conversion of amino acid to carbohydrate in the intact animal treated with adrenal cortical hormone (4) could well be due to an additional influence of the hormone on the kidney or some uninvestigated site.

The interpretation of the effect of adrenal cortical hormone on the oxygen consumption of liver slices is difficult, since the total oxygen consumption represents the sum of multiple oxidative reactions of which only a small portion may be influenced by the hormone. It is also probable that the nutritional state of the rats immediately preceding the experiment may play an important rôle in determining the proportion of oxidative processes which are affected by adrenal cortical hormone.

SUMMARY. In rat *liver slice*, adrenal cortical hormone increased the synthesis of carbohydrate from pyruvate or d-lactate but not from d-l-alanine or d-glutamate. Following a 24-hour fast the total carbohydrate content of the liver of adrenalectomized rats was lower than that of normals. The total carbohydrate content of the liver of normal 24-hour-fasted rats was increased significantly within one hour after the intraperitoneal injection of 5 ml. of adrenal cortical extract. In several instances changes in oxygen consumption of rat liver slices following adrenal cortical hormone therapy did not parallel the changes which were observed in the rate of carbohydrate synthesis.

CONCLUSION

Studies on rat liver slices indicate that the rate of synthesis of carbohydrate from pyruvate and d-lactate is markedly increased by adrenal cortical hormone.

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THE RATE OF GLUCOSE ABSORPTION FROM THE INTESTINE OF THE RAT

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The idea that the rate of intestinal absorption of glucose is independent of the amount present and time dates from the work of Cori (1). Subsequent investigators (2–7) failed to confirm his observations and found that glucose absorption decreased with time after the administration of the sugar and is dependent to some extent upon the amount of glucose available for absorption. The present study demonstrates that all of these conclusions as well as others in the literature are based upon observations made under certain *special conditions* and may have little bearing upon the rate of glucose absorption under ordinary circumstances.

METHODS. The present experiments were carried out with a uniform inbred stock of albino rats (8). With certain exceptions which are described elsewhere in this paper Cori's well known method (1) for studying the rate of absorption from the intestinal tract was used. During the extraction of residual glucose from the isolated tract after a given absorption period glucolysis was inhibited by the addition of sodium fluoride. Protein was removed from the intestinal tract extract by the use of zinc hydroxide precipitation (9) leaving a negligible amount of non-glucose reducing substances in the filtrates. Glucose determinations were carried out by the Shaffer-Somogyi method (10).

Glucose absorption rate under special conditions (forced feeding). Using essentially Cori's technique (1) in experiment 1 (table 1) results were obtained which support our earlier conclusion (4) that under these conditions the rate of absorption of glucose definitely falls off after the first hour. The dose of glucose and the concentration of the solution which was administered was similar for the rats in all groups. The length of the absorption period was the only variable.

In experiments 2 and 3 the absorption period was constant but the dose of glucose administered was varied. This was done in two ways. In experiment 2 the volume of solution administered was kept constant and the concentration of the glucose varied. Under these special conditions a relatively constant absorption coefficient was obtained, a result which supports Cori's contention that the rate of absorption is independent of

the amount of glucose present. However, this is true only under the special conditions of this experiment and is not a conclusion of broad applicability.

TABLE 1

Influence of time, dose and solution volume on the absorption coefficient for glucose under certain conditions of forced feeding

GROUP	TIME AFTER GLUCOSE	DOSE OF GLUCOSE*	BODY WEIGHT	BODY SURFACE	VOL. GLUCOSE SOLUTION†	CONC. GLUCOSE SOLUTION	ABSORPTION COEFFICIENT‡
Experiment 1.§ Influence of time							
	hours	*mgm.*	*grams*	*sq. cm.*	*cc.*	*per cent*	*mgm.*
1	1.0	876	142	3.08	2.0	43.8	116
2	2.0	876	141	3.07	2.0	43.8	96
3	3.0	876	140	3.06	2.0	43.8	87
4	4.0	876	141	3.07	2.0	43.8	80
5	6.0	876	141	3.07	2.0	43.8	77
Experiment 2.¶ Influence of glucose concentration and dose							
1	1.5	200	175	3.55	2.0	10.0	102
2	1.5	400	176	3.57	2.0	20.0	113
3	1.5	600	178	3.58	2.0	30.0	105
4	1.5	800	178	3.58	2.0	40.0	107
5	1.5	1000	178	3.58	2.0	50.0	118
Experiment 3.‖ Influence of variable concentration and dose							
1	1.5	200	178	3.59	0.4	50.0	54
2	1.5	400	179	3.61	0.8	50.0	73
3	1.5	600	180	3.62	1.2	50.0	96
4	1.5	800	189	3.74	1.6	50.0	99
5	1.5	1000	183	3.65	2.0	50.0	104

* Milligrams per 100 sq. cm. body surface.

† Cubic centimeters per 100 sq. cm. body surface.

‡ Milligrams per 100 sq. cm. body surface per hour.

§ Each group was made up of four female rats of approximately the same age. After fasting for 24 hours 2 cc. of 43.8 per cent glucose solution per 100 square centimeters body surface was given each rat at the beginning of the absorption period.

¶ Each group was composed of three adult male rats close to the same weight. After fasting for 40 hours the rats in each group were given different doses of glucose in the same volume of solution and the absorption rate observed for the ensuing 1.5 hours.

‖ Identical with experiment 2 except for the varying concentration of the glucose solutions which were administered.

When, as in experiment 3, variable doses of glucose were administered at the same concentration but in variable volume there was a very definite relation between the absorption coefficient and the dose of glucose administered. This confirms earlier observations (4, 5).

It would appear obvious that the nature of the results obtained in studying the rate of intestinal glucose absorption by Cori's method will depend on the special conditions under which observations are made. Our present interest concerns the relation of such results to the rate of glucose absorption under more normal conditions. The dietary habits of the rat are not such as ever to result in the influx into the stomach of a large volume of concentrated glucose solution during a few seconds. Furthermore, there is another point of importance. The maximum glucose absorption rates recorded by Cori or ourselves would hardly be sufficient to cover the basal energy requirement of the rat if glucose was absorbed at the maximum rate throughout the 24 hours. When this occurred to us we already had evidence that rats could be maintained on a diet consisting almost entirely of glucose. Since the rat normally confines its period of taking food almost entirely to the night time it was obvious that the glucose absorption rate under normal conditions must be considerably higher than it is generally believed to be.

Glucose absorption rate under "normal" conditions (voluntary feeding). During the course of experiments (11) in which rats were fed and fasted on alternate days the food intake on the feeding days was considerably greater than usual. The diet consisted almost entirely of sucrose (glucose and fructose) and if we assume the unlikely, that glucose (and fructose) absorption was going on continuously during the entire period that the rats were allowed food, the average absorption coefficient for the glucose (and fructose) in the diet would be 81. If, as is most likely, glucose absorption was proceeding during only a part of the feeding period this coefficient would be even higher. In an alternate feeding and fasting experiment similar to the one mentioned above but in which the rats were allowed access to the food cups for periods of 12 hours and fasted for 24 hours the appetite of most of the rats almost failed entirely, but a few of them ate sufficient to maintain their body weight after the usual initial weight loss. When these were sacrificed 3 hours after the end of the feeding period, allowing a total of 15 hours for possible glucose absorption, there was an insignificant amount of reducing substance in the gastro-intestinal tract. The diet was similar to the one we have used before (11) except for the substitution of glucose for sucrose. The results presented in table 2 show a great deal higher absorption rate for glucose than has been recorded in the various special and less normal conditions which have been used before.

The effect of protamine zinc insulin in increasing the appetite was utilized to study the rate of glucose absorption. Rats were removed from our stock diet, kept without food for 12 hours, and then allowed a diet, composed of the usual salt mixture 2, a syrupy rice polishing extract (rich in the vitamin B complex members) 3 and glucose 95, *ad lib.* The results comprise table 3. The glucose absorption coefficients are calculated on the

TABLE 2

Glucose absorption calculated over a 15 hour period at the end of an alternate fasting (24 hours) and feeding (12 hours) regime

Six rats in the group

Experiment 4

	MINIMUM	AVERAGE	MAXIMUM
Body weight (grams)	105	129	141
Body surface (sq. cm.)	252	290	306
Food intake (grams/rat/12 hrs.)	8.1	10.2	12.3
Glucose intake (grams/rat/12 hrs.)	6.7	8.4	10.1
Glucose absorption coefficient*	264	290	331

* Milligrams per 100 sq. cm. body surface per hour.

TABLE 3

Rate of glucose absorption by rats on a largely glucose diet and given protamine zinc insulin (PZI)

	DAYS					
	1	2	3	4	5	6
Experiment 5, male						
Body weight (grams)	287	285	281	274	269	265
PZI (units)	10	5	5	0	0	0
Glucose (grams/24 hours)	27	32	24	14	21	20
Glucose absorption coefficient*	228	272	206	122	185	178
Experiment 6, male						
Body weight (grams)	307	302	295	293	289	286
PZI (units)	10	7	4	0	0	0
Glucose (grams/24 hours)	28	22	24	14	15	20
Glucose absorption coefficient*	226	179	198	116	126	169
Experiment 7, average of 4 males						
Body weight (grams)	339	329	321	310		
PZI (units)	3	3	3	4		
Glucose (grams/24 hours)	40	38	34	44		
Glucose absorption coefficient*	302	293	267	353		
Experiment 8, average of 2 females						
Body weight (grams)	169	162	154	149		
PZI (units)	4	4	0	0		
Glucose (grams/24 hours)	29	28	17	22		
Glucose absorption coefficient*	349	347	217	288		

* Glucose as milligrams absorbed per 100 square centimeters body surface per hour.

assumption that absorption proceeded evenly over the 24 hour periods. This was probably not the case and it is likely that the coefficients were at times much higher. But those which have been calculated are far higher than has hitherto been thought to be possible. Although it seems to be an unlikely possibility it may be argued that the high absorption rates for glucose in these experiments are due to the effect of the protamine zinc insulin. Naturally, this is true in so far as this agent is responsible for the large glucose intakes, but with our present knowledge there is little reason to believe that it has any influence on the rate at which glucose passes from the intestine into the blood stream.

It was suggested to us by Professor Drury that an excellent way in which to get rats voluntarily to take large quantities of glucose was to place them in a low environmental temperature such as a refrigerator, and offer them a 50 per cent glucose solution to drink. The glucose absorption period was taken from the time they were placed in the cold until three hours after removing the glucose solution, at which time we knew by examining the contents of their intestinal tracts that absorption was complete. Although it is improbable that absorption was in progress throughout this period absorption coefficients for glucose as high as 564, 520 and 435 mgm. were found in female rats weighing 200 to 234 grams when absorption was calculated in this manner.

SUMMARY

The rate of absorption of glucose from the intestine of the rat under the conditions of forced feeding with strong glucose solutions which are generally used depends upon the amount and concentration of the sugar. When the dose of glucose and the concentration are kept constant the absorption coefficient (milligrams absorbed per 100 square centimeters body surface per hour) decreases with time after administration. Variable doses administered in a fixed volume gave relatively constant absorption coefficients (102–118) over a single absorption period. When variable doses of glucose were administered at the same concentration but in variable volume the absorption coefficient was related to the dose of glucose.

Under normal or at least voluntary feeding conditions absorption rates several times higher than those previously reported were found. By concentrating the period of eating through a prior period of fasting and offering a diet composed largely of glucose, absorption coefficients of 300 mgm. were obtained. By giving glucose alone to eat and increasing the appetite with protamine zinc insulin injections as much as 200 to 300 mgm. of glucose per 100 sq. cm. body surface was absorbed each hour. A very cold environment increased the appetite so much that 50 per cent glucose was taken in quantities large enough to give absorption coefficients of over 500.

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THE INFLUENCE OF A DIET WITH A HIGH PROTEIN CONTENT UPON THE APPETITE AND DEPOSITION OF FAT

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There is an almost complete absence of the usual fat deposits in rats which have been maintained for a long time on a diet high in protein and containing no preformed carbohydrate (1). Whether this is due primarily to a disturbance in the fat storing mechanism or in the appetite, it is necessarily an appetite effect. The data which aroused our interest in this subject (1) are not such as to suggest an appetite effect but if the food intake had been falling off in comparison with that of the rats on the diet containing more moderate amounts of protein the body weight would have been decreasing at the same rate. Since the food intake was recorded in relation to body size any influence of the diet on the appetite would not be obvious. Placing the food intake on a body size basis is essential for some purposes (2) but becomes undesirable when the appetite is the point under investigation. The experiments reported here were undertaken to determine the influence of the protein content of the diet on the appetite and secondarily the deposition of body fat in the rat.

METHODS. Albino rats of known age were used. They came from the same strain which has been maintained for the past 18 years (3). The special diets which are detailed in table 1 were freshly made every 10 days. The rats were caged in groups of 3 to 6 and weighed at 2 to 4 day intervals. Group caging made it necessary to remove from an experiment any rat whose change in body weight was greatly different from that of its mates. The food cups were weighed every second day. At the end of the feeding periods the etherized rats were finely ground in a meat chopper and samples of the well mixed hash used for fat and water analysis. Water was determined by drying the sample to constant weight at 100°C. in a vacuum oven. Fat represented fatty acids plus unsaponifiable lipids determined in the usual manner (4).

RESULTS. Those experiments which are pertinent to the object of this study are summarized in table 2. The observations on the appetite as such require no explanation. However we are also interested in the amount of deposit fat. In a given experiment the latter will bear a relation to the appetite as measured by the average daily food intakes of the

various groups only if the energy expenditures of the different groups are similar. This must be the case for in a general way the body fat content and appetite tend to move in the same direction.

Protein intake and appetite. Variations in body weight and food intake for two day intervals throughout the feeding period of experiment 1 are presented in figure 1. These were adult rats and all of them weighed less at the end of the experiment than at the beginning. Such a weight loss

TABLE 1

	DIET NUMBER																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Casein*	15	30	50	75	20	70	20	50	50	20	84	29	20	22	28	30	70
Sucrose†	60	45	25	0	50	0	0	20	0	40	0	0	50	25	0	24	0
Salt mixture‡	4	4	4	4	5	5	5	5	5	5	4	6	4	5	6	6	5
Yeast§	6	6	6	6	5	5	5	5	5	10	8	11	6	7	9	6	5
Cod liver oil¶	2	2	2	2	5	5	5	5	5	5	4	6	2	2	3	6	5
Corn oil‖	3	3	3	3									3	4	5		
Hydrogenated vegetable oil**	10	10	10	10	15	15	37	15	24	20	0	48	15	35	49	21	15
Cellulose††							28		11							7	
Calories per gram	4.6	4.6	4.6	4.6	4.8	4.8	4.8	4.8	4.9	5.0	3.8	6.3	4.9	5.9	6.5	4.7	4.7
Per cent of calories as:																	
Protein	16	27	45	70	19	62	19	45	45	21	96	20	20	18	18	25	60
Fat	30	30	30	30	38	38	81	38	55	47	4	80	38	65	82	54	40
Carbohydrate	54	43	25	0	43	0	0	17	0	32	0	0	42	17	0	21	0

* Vitamin free casein prepared by the Casein Company of America.

† Cane sugar product, rather finely granulated and known to the grocery trade as "Bar" sugar.

‡ Osborne and Mendel's (5) mixture obtained from The Harris Laboratories, Tuckahoe, N. Y.

§ Brewer's yeast obtained from (Strain G) Anheuser-Busch.

¶ Tested commercial brand of Norwegian origin.

‖ Cooking oil marketed under name of Mazola.

** Crisco.

†† Cellu Flour, Chicago Dietetic Supply Company.

is common in adult rats when they are moved from sawdust bedding to cages with wire mesh bottoms. The group which received the high protein diet (4, expt. 1, table 2) ate less than those on the control diet (1, expt. 1), lost more weight and had less deposit fat than the controls. The difference in food intake and body weight on the two diets is shown even more strikingly in the group in experiment 1 (fig. 1) in which the feeding of these two diets was alternated. The high protein diet always led to a decrease in the appetite and a loss in body weight.

TABLE 2

	EXPERIMENT NUMBER																
	1		2				3					4			5		
	Diet no.																
	1	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Days on diet	68	68	54	54	54	54	48	48	48	48	48	35	35	35	54	54	54
Initial body weight (grams)	236	236	71	71	71	71	218	217	217	218	217	138	136	135	240	241	241
Final body weight (grams)	234	222	179	207	211	154	297	250	271	270	271	217	168	213	325	352	354
Body "fat" (per cent)	15.4	9.3	9.6	11.2	10.8	8.3	13.6	6.2	13.1	10.3	11.0	10.4	8.2	14.9	17.8	21.1	20.0
Body water (per cent)	62.7	64.1	64.8	65.6	64.6	64.8	59.6	60.8	59.1	59.1	59.5	63.1	54.0	62.3			
Food intake during feeding period in grams per rat per day	8.6	8.1	7.7	8.2	8.7	5.6	10.5	8.7	9.8	9.7	9.7	9.0	10.1	6.9	11.6	10.2	9.4
In calories per rat per day	40	37	35	38	40	26	50	42	47	47	47	45	38	42	57	61	61

Experiment 1: 6 female rats in each diet group. See figure 1.

Experiment 2: 8 male rats in each group. See figure 2.

Experiment 3: 6 male rats in each group. In this experiment fat and water determinations were made on the carcass minus all viscera.

Experiment 4: 6 male rats in each group. They were 65 days old at the start of the feeding period.

Experiment 5: 6 male rats in each group. They were 95 days old at the start of the feeding period.

In young growing rats (expt. 2, table 2) increases in the percentage of calories as protein led to an increase in the food intake, more body fat, and a higher body weight until a diet with a very high protein content and free of preformed carbohydrate was offered. These differences in the growth rate have been noted before (6). The relation of the appetite to the protein content of diets containing preformed carbohydrate will be considered elsewhere. In figure 2 the body weight and food intake on high (no. 4) and low protein (no. 1) diets have been compared throughout the feeding period. A third group in figure 2 in which the diets were alternated demon-

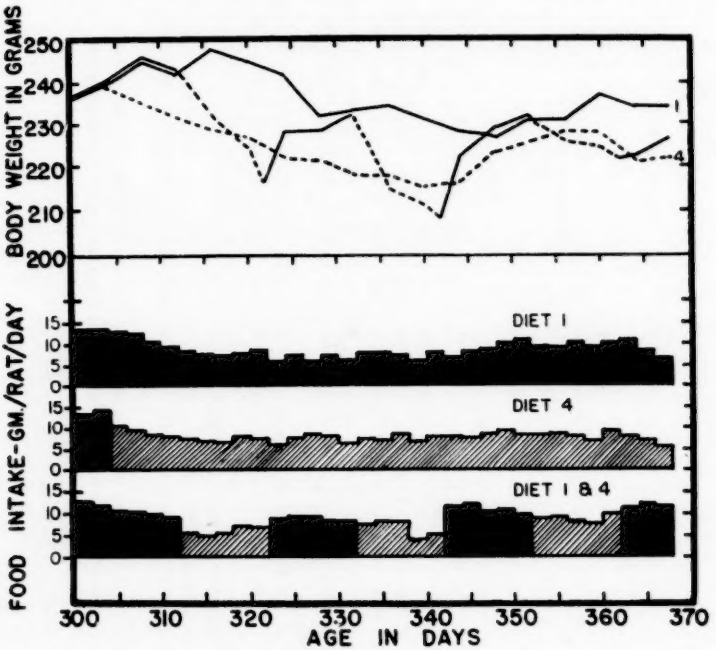

Fig. 1. Experiment 1. Comparison of the influence of low and high protein diets upon the appetite in adult rats.

strates the decreased appetite and body weight loss on the high protein diet in a spectacular manner.

The results reported here were obtained on diets in which casein served as the protein. Similar results have been obtained when commercial dried blood albumen was used as the source of protein. The addition of 0.05 per cent thiamin chloride to the diets did not alter the results. The diets used in experiments 1 and 2 contained the same percentage of their calories as fat. The higher protein levels were provided at the expense of carbohydrate so that the high protein diet which had the deleterious influence

on the appetite was devoid of preformed carbohydrate. It was therefore necessary to determine what part the removal of carbohydrate calories

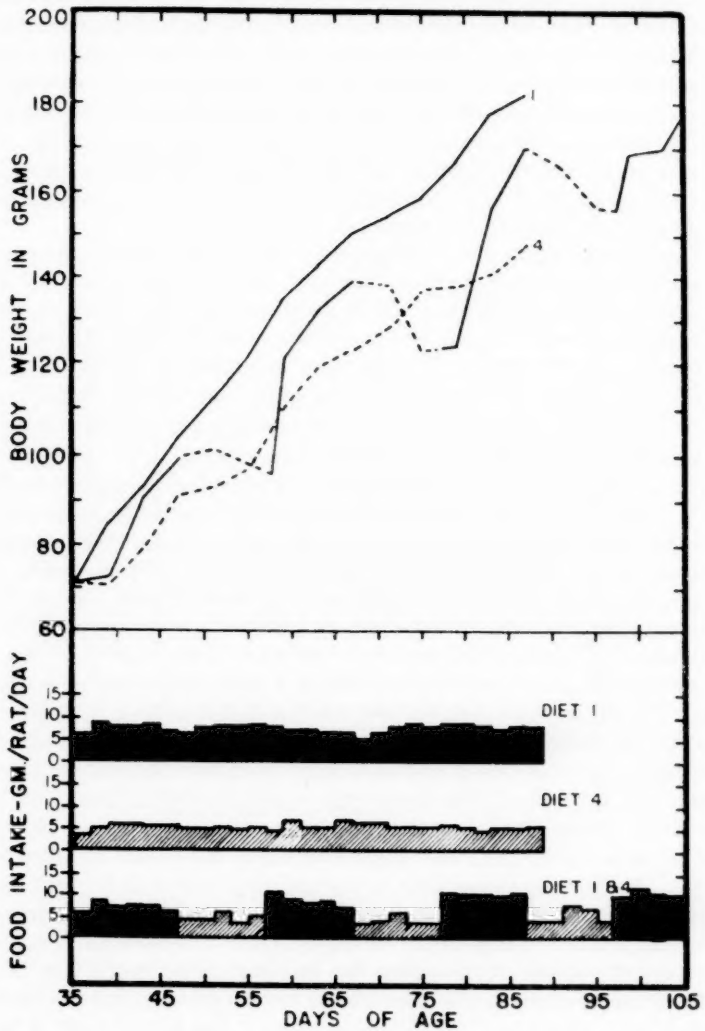

Fig. 2. Experiment 2. Comparison of the influence of low and high protein diets upon the appetite in young growing rats.

from the diet played in the reduced appetite resulting from a high protein content.

Carbohydrate lack in the diet and appetite. In experiment 3 diet groups 5 (low protein) and 6 (high protein) again demonstrated what had been shown in the preceding experiments. Diet 7 had the same protein content as diet 5 but was carbohydrate free as was diet 6, the carbohydrate calories being made up by fat. This diet was made rather bulky by the addition of cellulose to maintain a constant caloric value in relation to weight but the appetite was essentially the same as in the carbohydrate containing diet group (no. 5). Diet groups 8 and 9 bore on the same point and gave further evidence that the absence of carbohydrate from the diet containing the highest protein content was not responsible for the reduction in appetite and subsequent lessening of body fat.

Experiments 4 and 5 were designed to examine the relative influence of protein excess and carbohydrate lack upon the appetite without the complication of bulking cellulose. This introduced another factor, variation of energy value with weight, but in our experience it is less important than a high degree of variation in energy value in relation to the diet volume or bulk. The results of experiment 4 supported those found in the preceding experiment and experiment 5 is even more striking in demonstrating the failure of carbohydrate lack to influence the appetite.

Glucose capacity of the diet and appetite. A possible physiological explanation of why an excessive protein intake may reduce the appetite rests in the glucogenic capacity of such a diet. There is evidence that the marked increase in the appetite and body fat stores produced by protamine zinc insulin administration (7, 8) is a function of a reduced blood sugar level (8). The organism receives glucose from carbohydrate as such and from the catabolism of protein (at least 58 per cent may form sugar). Conn and Newburgh (9) have found that protein is a more advantageous source of sugar for the diabetic than carbohydrate and that chronic hypoglycemia (10) is best treated with a high protein diet because the glucose yielded by protein is available slowly and at a more even rate than in the form of carbohydrate itself which is stored quite rapidly and thus disappears from the blood stream. A high protein diet might conceivably reduce the appetite by maintaining the blood sugar at a higher level than do other foodstuffs. Pollack and Dolger (11) have found that protein is the best source of carbohydrate for preventing nocturnal hypoglycemia in diabetic patients receiving protamine zinc insulin therapy. These considerations suggested an indirect method of attacking the problem by a comparison of the appetite effect of protamine zinc insulin in rats on high and low protein diets.

Figure 3, in which are plotted the averages of experiment 6, is a typical example of several experiments of the same type. There were six female rats in each group. The insulin was not without influence upon the appetite and body weight of the rats receiving the high protein diet but it had

much less effect than on the rats whose diet contained a lower protein content. The control rats on diet 1 consumed an average of 9.4 grams while the insulin treated rats on this diet ate 11.4 grams of food per day. On the high protein diet (no. 4) the corresponding figures were 9.0 and 9.6 grams per day respectively. When the rats were 230 days old and had been receiving insulin for two days, two of those on diet 4 died in hypoglycemia. Another one of this group succumbed in the same manner 10

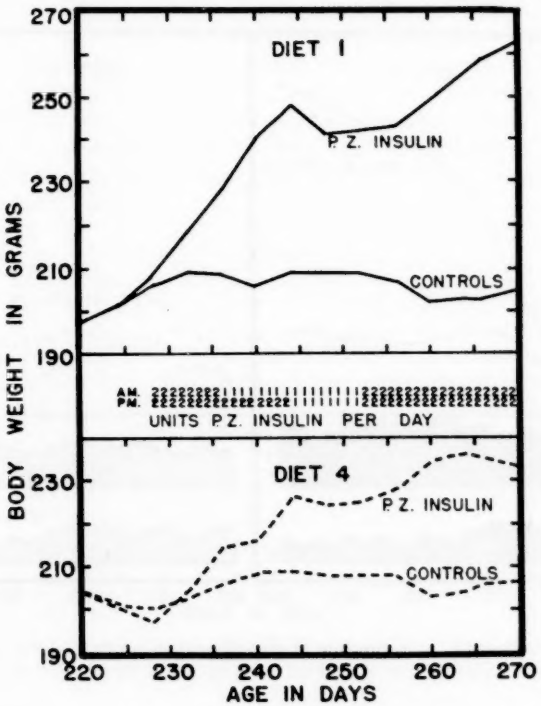

Fig. 3. Experiment 6. Comparison of the influence of protamine zinc insulin upon the appetite of rats on low and high protein diets which are isocaloric.

days later. There was no evidence of a symptom producing hypoglycemia among the insulin treated rats on diet 1 at any time. It should be pointed out that while diets 1 and 4 were calorically equivalent, diet 1 had 69 per cent available carbohydrate in comparison with only 45 per cent in diet 4. An insulin experiment was therefore carried out with low (diet 16) and high (diet 17) protein diets containing isoglucogenic quantities of protein and carbohydrate (on the assumption that only 58 per cent of protein may be

converted to glucose). There were five male rats in each group. The insulin treated rats each received 2 units twice daily of protamine zinc insulin (Squibb's U-40 preparation). The results for this experiment (expt. 7) are pictured in figure 4. The difference in the influence of the insulin upon the appetite of rats receiving low and high protein diets is even more obvious than in experiment 6. None of the rats showed symptoms of hypoglycemia at any time. These results suggest that the marked difference in appetite between rats on a low protein and a high protein

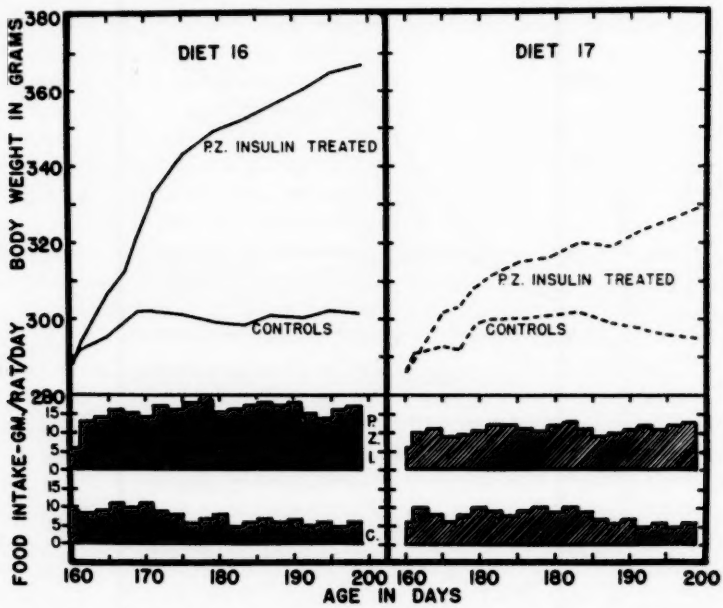

Fig. 4. Experiment 7. Comparison of the influence of protamine zinc insulin upon the appetite of rats on low and high protein diets which are isocaloric and isoglucogenic.

carbohydrate free diet may be a result of the maintenance of the blood sugar at a higher level from carbohydrate derived from protein than when it is fed as such. A preceding high protein diet maintains the liver glycogen (12, 13) and blood sugar levels (13) during fasting better than a lower protein intake.

SUMMARY

When the carbohydrate in an adequate synthetic diet is replaced with protein (casein) the appetite of albino rats is definitely less than on the carbohydrate containing diet. Alternation of the diets demonstrates the

difference in appetite very clearly. Rats offered such diets for a time show marked differences in body weight and fat content, those on the high protein diet having less deposit fat.

The reduced appetite on a high protein diet is due to the protein content and not lack of carbohydrate as such, for replacement of the carbohydrate calories with fat instead of protein does not result in the same marked reduction in the appetite and body fat.

The administration of protamine zinc insulin produces much less of an increase in the appetite with a diet high in protein and containing no carbohydrate than with an isoglucogenic diet containing less protein and a large portion of the calories as carbohydrate. Since protamine zinc insulin appears to increase the appetite by reducing the blood sugar level this suggests that a high protein, carbohydrate free diet may decrease the appetite by better maintaining the blood sugar level, glucose from protein being formed slowly and therefore more evenly available than when fed as carbohydrate.

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OBSERVATIONS ON THE LOCALIZATION OF THE RECEPTOR AREA OF THE BAINBRIDGE REFLEX[1]

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Bainbridge (1) in 1915 observed that a rise in venous pressure following intravenous injection of saline and defibrinated blood was responsible for a cardiac acceleration operating from the venous side of the right heart. The acceleration was thought to have been brought about partly through loss of vagal tone and partly through increased accelerator tone. This mechanism was believed to cause the cardiac acceleration incident to muscular exercise and its concomitant increased venous inflow and higher venous pressure.

Sassa and Miyazaki (2) confirmed the findings of Bainbridge. Objections, however, may be raised to the methods used by both groups. No consideration was given by Bainbridge to the changes of the circulation in the lungs and in parts of the heart other than the venous side. Sassa and Miyazaki, by using rubber balloons to raise the pressure in the venae cavae and right auricle, introduced other modifications in circulation to which the change in heart rate could be attributed. More convincing confirmation of Bainbridge's interpretation was presented by Anrep and Segall (3) who, in a series of well-controlled experiments on innervated heart-lung preparations, found the cardiac acceleration to occur with an increase of the venous return. However, since they observed in many experiments a definite acceleration with little or no rise in the venous pressure, they concluded that it was premature to regard the venous pressure elevation as the factor responsible for the reflex.

The acceleration of the heart does not always appear. Thus Wiggers and Katz (4) found absence of heart rate changes on varying the venous pressure in many of their experiments with anesthetized intact animals. Also, DeGraff and Sands (5) observed an increased heart rate in only half the animals similarly studied, and one-third of all the animals showed this acceleration even after the vagi were severed bilaterally. They concluded,

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therefore, that it was a less constant mechanism than the work of Bainbridge would indicate.

There is sufficient discrepancy in previous results to question the simple interpretation usually given to the so-called Bainbridge reflex. It was therefore decided to reinvestigate this subject in a manner that would exclude objections that might be raised as to methods and anesthesia used in the preceding studies.

Chloralosane was employed as an anesthetic in those of our experiments in which anesthesia was desired. This was suggested by Heymans (6) who has shown that chloralosane does not depress the cardiac reflexes as do many of the other anesthetics. Secondly, the experiments in which distention of the vena cava or right auricle was performed utilized a by-pass arrangement so that distention had little or no effect on the circulation such as occurred in Sassa and Miyazaki's experiments. And thirdly, experiments were performed on unanesthetized closed-chested animals, some of which were trained, to study the effects of an increased venous return.

In addition, the effect of distention of that part of the superior vena cava where Nonidez (7) has found typical pressor receptors was noted both in chloralosane-anesthetized and in trained and untrained unanesthetized dogs. This was done by special cannulae so that no interruption in the flow of blood was produced. Histologically, these subendothelial end-organs found by Nonidez in the intrapericardial portions of all veins entering the heart resembled those of the carotid sinus and aorta, and he concluded that the mechanical excitation of the subendothelial nerve end-organs during increased venous pressure initiated the accelerating reflex.

Methods. The majority of experiments were performed upon unanesthetized dogs to ensure that the reflex was not altered by anesthesia. Three of these dogs were untrained and consequently $\frac{1}{2}$ grain morphine sulphate was administered subcutaneously to obtain complete relaxation and to increase vagus tone. All the other animals were trained to lie quietly.

The venous and arterial blood pressures were recorded by means of Hamilton manometers (8) and from these the heart rate was determined. In the determination of the heart rate, from 20 to 40 beats were included in a measurement. The product of sixty times the number of beats included divided by the duration of the interval in seconds gave the rate in beats/min. Care was taken to include one or two respiratory cycles when marked sinus arrhythmia was present. When a non-respiratory arrhythmia was found the entire cycle of this arrhythmia was included in the measurements. The few experiments in which the heart rate showed a noticeable progressive change between the control period before and after the experimental procedure were discarded. When vagotomy was de-

sired, it was performed in anesthetized animals by section in the neck. Sympathectomy, also performed in anesthetized animals, was accomplished by removing the stellate and upper three sympathetic ganglia, bilaterally, in the open chest.

All injections of fluid were made at body temperature in the unanesthetized animals, using rectal temperature as the guide and placing the solutions in a constant temperature box for the purpose. In the anesthetized animals with open chest, this was accomplished by placing the solutions in the same constant temperature box in which the animal was kept. The solutions were injected by gravity and under pressure at a rate of from 260 to 800 cc./min. For the most part the solution injected was mammalian Ringer's, but in several experiments with open-chested dogs whole heparinized blood was used instead.

Fig. 1. A, photograph of modified Morawitz cannula used in open-chested dog to distend root of superior vena cava without obstructing circulation. The photograph shows the cannula with the distended balloon at its end in the position used when cannula is in place. The balloon is inflated through an air line extending along the outside of cannula. The lumen of cannula is thus always of constant size whether balloon is inflated or not.

B, photograph of umbrella-ribbed cannula. Photograph shows ribs extended. This cannula is inserted via the external jugular vein. The expansile ribs at lower end can be raised and lowered by means of a screw arrangement at the opposite end. The C clamp is applied to keep the ribs unexpanded during insertion. Side arm is used to inject fluids and to permit recording of venous pressure at root of superior vena cava.

Distention of the superior vena cava was performed either by means of a modified Morawitz cannula (fig. 1A) or another specially designed cannula with an umbrella-ribbed expansile end (fig. 1B).[2] The Morawitz-like cannula was used in open-chested chloralosanized animals only, with the blood rendered non-coagulable with heparin. The cannula was inserted via the distal part of the intrathoracic superior vena cava or inferior vena cava, with the expansile (balloon) tip placed at the junction of the superior vena cava and the right auricle as determined by visual control. Care was taken to avoid injury to nerve pathways coursing along the superior vena cava. The cannula with the umbrella-ribbed expansile tip was employed in closed-chested unanesthetized animals, the blood of which had also

[2] We are indebted to Dr. K. Jochim for his help in designing this cannula. Both cannulae were constructed by Mr. S. F. Gaddas.

been rendered non-coagulable with heparin. The cannula was inserted via the right external jugular vein under local procaine anesthesia, and the position of the cannula at the junction of the superior vena cava and right auricle was controlled fluoroscopically and checked at autopsy in each case. Absence of damage to the veins and the effectiveness of the distention were also checked postmortem. The construction of both cannulae was such as to permit local distention without any appreciable interference with blood flow. The umbrella-ribbed cannula was designed with a side-arm which permitted its use as a channel for injection of fluid intravenously.

In some open-chested dogs under chloralosane anesthesia, a special arrangement was established to by-pass the right auricle and the major veins which entered it. For this purpose a cannula was inserted into the lateral wall of the right ventricle and connected without interrupting the circulation to cannulae in the superior and inferior venae cavae, the coronary sinus and the azygos vein. In this way little or no blood entered the right auricle and the adjacent venae cavae. The procedure was carried out with care not to interfere with the innervation of the heart. In this preparation an increase in the venous return would not affect the pressure in the isolated auricle and proximal parts of the venae cavae and, furthermore, the auricle could be distended by a balloon without interfering with the circulation of blood.

Results and their interpretation. *1. Effect of increased venous return.* A series of 16 experiments was carried out on 12 animals, all but two of which were performed on unanesthetized dogs. The two exceptions were performed on open-chested dogs under chloralosane anesthesia. The results are summarized in table 1 and a typical result on an unanesthetized animal is illustrated in figure 2 and the original record in figure 3. No pulse acceleration occurred in the chloralosanized dogs (one of these had had the heart completely denervated at the time of injection), and in two trials in unanesthetized dog 14. All other experiments showed a definite temporary cardiac acceleration, the peak of which came about 30 to 40 seconds after the start of the injection and the acceleration lasted several minutes.

In those experiments in which the rate of injection was rapid, the arterial pressure rose during the injection and accompanying this rise there was often a fleeting cardiac slowing early in the injection period (fig. 4). This slowing is probably a depressor reflex originating from the presso-sensitive end-organs of the arterial buffer nerve mechanism. This may also explain the secondary fleeting lowering of the blood pressure below the control level so often seen (figs. 2 and 3). However, part of this drop may be due to the decrease in blood viscosity by the Ringer's solution which on passing through the periphery lowered the peripheral resistance. The absence of these transitory effects when the rate of injection was slow (viz., table

1, dogs 17 and 18) would fit with either explanation. Aside from this transitory drop, the arterial blood pressure remained elevated for a time.

The venous pressure rose over 400 mm. of blood during the injection and was also elevated for a period after the injection (figs. 2, 3 and 4). These pressure elevations indicate that the pulmonary vessels are also distended, as well as the heart chambers. Any one of these regions therefore could be the site initiating the Bainbridge reflex.[3] That some other locality than the large veins and right auricle may be the focus initiating

TABLE 1

Effect of increasing venous return by intravenously injecting mammalian Ringer's solution at body temperature

DOG NUMBER	AMOUNT INJECTED	DURATION OF INJECTION	MAXIMUM HEART RATE CHANGE FOLLOWING INFUSION	RESPONSE CONSIDERED
	cc.	*sec.*	*beats/min.*	
5*	150	7	0	0
6*†	150	39	4	0
9‡	200	31	21	+
10‡	200	24	66	+
11‡	200	42	18	+ (?)
12§	200	48	33	+
13§	200	14	34	+
14§	200	23	2	0
	200	14	6	0
15§	200	19	42	+
16¶	200	17	78	+
	200	15	70	+
	200	16	14	+ (?)
	200	15	36	+
17§	200	84	78	+
18§	200	96	46	+

* Open-chested dog under chloralosane anesthesia.

† Heart completely denervated.

‡ Partially trained unanesthetized dog under ½ grain morphine sulphate.

§ Trained unanesthetized dog.

¶ Trained unanesthetized dog; the injections of Ringer's solution were made while animal under ½ grain morphine sulphate.

the cardiac acceleration is suggested by the presence of an acceleration of the heart following increased venous pressure in experiments where the exclusion of the venae cavae and right auricle by the use of a by-pass (table 2) would remove the possibility of stimulation of end-organs in these regions. Since, in these experiments, the heart chambers and pulmonary vessels were distended so that the pulmonary pressure rose, the possibility

[3] Care in keeping the temperature of the injected solution at body temperature excludes the possibility that this is a thermal effect.

is suggested that stimulation of end-organs here may cause, or at least contribute to, the Bainbridge reflex.

The occurrence of negative experiments as far as the Bainbridge reflex is concerned we cannot explain.

We conclude from these results that the Bainbridge reflex does occur in unanesthetized animals, but not without occasional omission. Further,

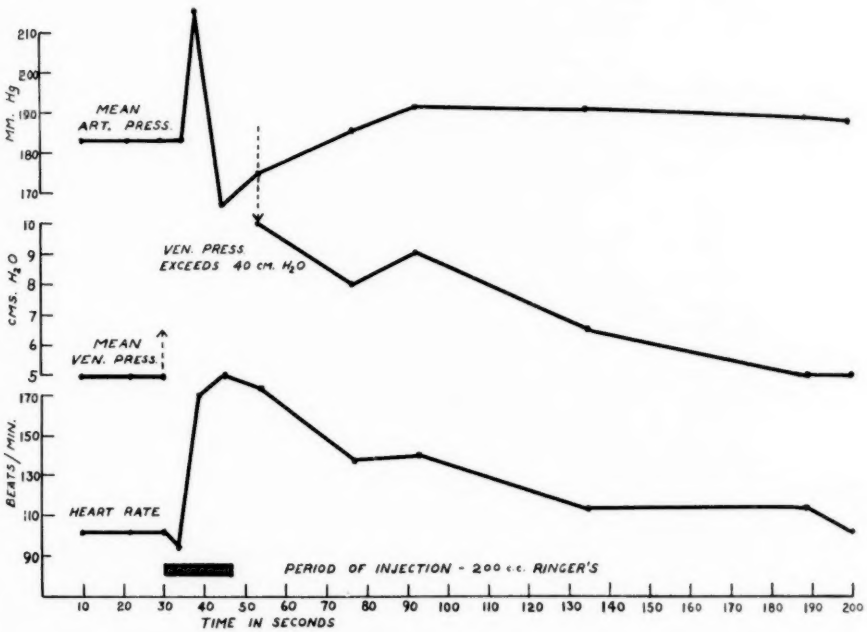

Fig. 2. Chart showing changes in mean arterial pressure (upper), mean venous pressure (middle) and heart rate (lower curve) upon increasing the venous return. Note extreme cardiac acceleration resulting from elevation of the venous pressure. (Injection made at body temperature in interval indicated by block.)

reflexes originating in the pulmonary vessels or in the heart chambers other than the right auricle are probably involved, at least in part, in its initiation.

2. Effect of distention of the superior vena cava and right auricle. It is significant that while distention of the heart exclusive of the superior vena cava and right auricle leads to cardiac acceleration, distention of these latter chambers alone, without affecting the flow of blood in the circulation had no apparent effect on the heart rate in four trials on one preparation with the right auricle and venae cavae by-passed (table 3). This would

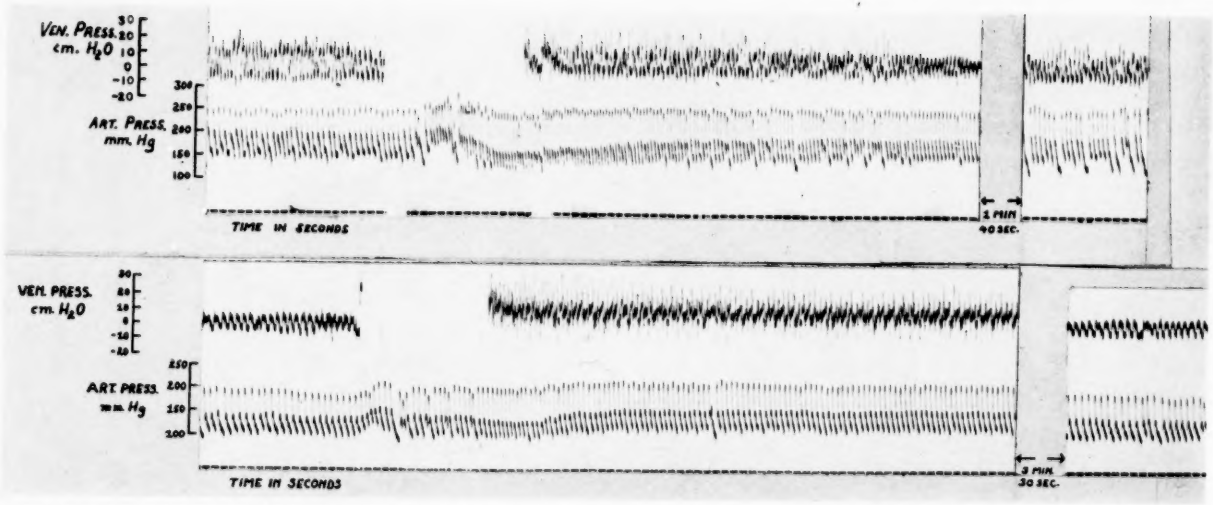

Fig. 3. (Upper.) Simultaneous optically recorded curves showing changes in venous pressure (upper) and arterial pressure (lower) resulting from increasing the venous return with 200 cc. of Ringer's solution (at body temperature). Venous pressure recorded at junction of superior vena cava and right auricle. Arterial pressure determined from femoral artery. Heart rate obtained from arterial pressure curve. Injection period shown by period in which venous pressure is off the record.

Fig. 4. (Lower.) Simultaneous optically recorded curves of venous and arterial pressures showing changes following intravenous injection of 200 cc. Ringer's solution. Note transient cardiac slowing at peak of injection, followed by the typical cardiac acceleration. Conventions as in figure 3.

confirm the view that the cardio-accelerator reflex originates elsewhere than in these regions. The positive results reported by Sassa and Miyazaki (2) must therefore be attributed to the disturbances in circulation produced in their balloon distention which our arrangement avoided. Their experiments, therefore, cannot be assumed to support the presence of a Bainbridge reflex or of its origin in the right auricle and superior vena cava.

3. Effect of distention of the superior vena cava at its junction with the right auricle. This was first performed on chloralosanized dogs using the modified Morawitz cannula (fig. 1A). The results are summarized in

TABLE 2

Effect of increasing venous return in anesthetized dogs with right auricle and venae cavae by-passed

DOG NUMBER	AMOUNT INJECTED	DURATION OF INJECTION	MAXIMUM HEART RATE CHANGE FOLLOWING INFUSION	RESPONSE CONSIDERED
	cc.	*sec.*	*beats/min.*	
2	100*	16	66	+
	100*	16	37	+
3	100†	5	68	+
	100†	3	65	+
4	100†	2.5	4	0
	150†	3	2	0
	100†	6	10	0
	100†	4.5	6	0
	150†	11	28	+
	100†	12	64	+

All injections at body temperature.
* Mammalian Ringer's solution used.
† Whole heparinized blood used.

table 4. In two of the four dogs, nos. 6 and 8, no heart rate changes were observed on repeated distention. In dog 5, however, repeated distention gave no heart rate change at first; but on the fifth trial a definite cardiac acceleration was noted which was also obtained on distention after one and then both vagi were severed. In dog 7, on the other hand, distention caused a cardiac slowing when the nerves were intact which disappeared after bilateral vagotomy. These results suggest that stimulation of the end-organs located at the root of the superior vena cava can cause heart rate changes, but there may on occasion be a slowing rather than an acceleration depending upon circumstances still unknown. The slowing reflex depends upon the vagus since it disappears when the vagi are severed.

TABLE 3

Effect of distention of rubber balloon in superior vena cava and right auricle in dog with these chambers by-passed

DOG NUMBER	MAXIMUM HEART RATE CHANGE DURING INFLATION	RESPONSE CONSIDERED
	beats/min.	
4	−1	0
	−8	0
	0*	0
	6*	0

* Premature beats appeared at start of inflation.

TABLE 4

Effect of distention of superior vena cava at junction with right auricle by means of inflating a balloon on a modified Morawitz cannula in open-chested dogs anesthetized with chloralosane

DOG NUMBER	MAXIMUM HEART RATE CHANGE DURING INFLATION	RESPONSE CONSIDERED	REMARKS
	beats/min.		
5	5	0	
	4	0	
	6	0	
	6	0	
	40	+	
	36	+	After left vagotomy
	44	+	After left vagotomy
	47	+	After bilateral vagotomy
	22	+	After bilateral vagotomy
	22	+	After bilateral vagotomy
6	2	0	Distention also performed 7 times after complete denervation of the heart with no heart rate change
	−1	0	
	0	0	
	−5	0	
	4	0	
7	−19	R*	
	−43	R*	
	1	0	After bilateral vagotomy
	−4	0	After bilateral vagotomy
	4	0	After bilateral vagotomy
	−2	0	After bilateral vagotomy
8	10	0	
	4	0	
	−2	0	After bilateral sympathectomy
	−1	0	After bilateral sympathectomy
	−2	0	After complete denervation of heart
	5	0	

* The response here was definitely the reverse of cardiac acceleration.

TABLE 5

Effect of distention of superior vena cava at junction with right auricle by means of a special umbrella-ribbed cannula inserted under local procaine in unanesthetized dogs

DOG NUMBER	VARIATIONS IN CONTROL HEART RATE				MAXIMUM HEART RATE CHANGE DURING DISTENTION	RESPONSE CONSIDERED
	Maximum during respiratory arrhythmia (instantaneous rates)	Maximum in different control periods in course of experiment (average rates)	Maximum during periods of non-respiratory arrhythmia (average rates)	Average duration of non-respiratory arrhythmic cycle		
	beats/min.	*beats/min.*	*beats/min.*	*sec.*	*beats/min.*	
9*	13	6	none present		4	0
					−6	0
					−12	0
					−4	0
10*	16	10	none present		0	0
					8	0
					4	0
11*	57	10	15	16	8	0
					4	0
					0	0
					0	0
12	75	36	15	16	6	0
					2	0
					0	0
					27†	+
					12	0
13	80	70	none present		0	0
					−6	0
					0	0
					−3	0
14	none present	24	29	14	0	0
					12	0
					2	0
					6	0
15	36	54	51	28	0	0
					10	0
					0	0
16	82	12	none present		0	0
					3	0
					6	0

* Animal under ½ grain morphine sulphate.

† Animal became excited during distention period and showed tachypnea and dyspnea.

The cardiac acceleration apparently does not. The absence of heart rate changes following distention of the superior vena cava may thus not be the absence of a reflex but rather the neutralization of two oppositely acting reflex effects.

Since conditions under anesthesia are different from those in the absence of anesthesia, these experiments were repeated in unanesthetized animals with the umbrella-ribbed cannula (fig. 1B). The results of the 30 experiments upon 8 animals in which the root of the superior vena cava was distended by the expansile ribs of the cannula are shown in table 5 and

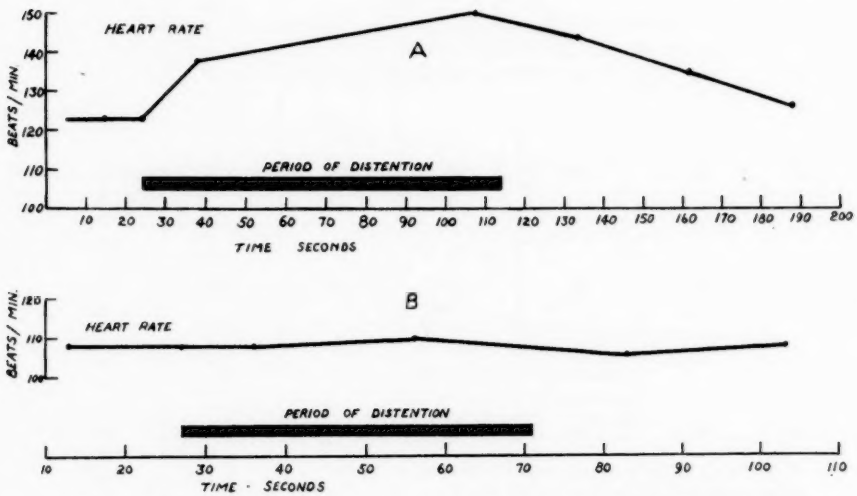

Fig. 5. Chart showing effect upon heart rate of distention of root of superior vena cava by umbrella-rib cannula inserted in unanesthetized dogs under local procaine anesthesia. A. The cardiac acceleration obtained in only one experiment. B. Typical negative effect of distention obtained in other 29 experiments.

illustrated in figure 5. Except for one experiment in which a cardiac acceleration occurred (fig. 5A), all the others were negative (fig. 5B).

Care was taken in these experiments to measure properly the heart rate changes since, as shown in table 5, there are variations in the heart rate. Some are respiratory in origin and can be marked in degree as the first column shows, the results being given in instantaneous heart rate, the reciprocal of individual cycle lengths. Besides there are longer cyclic fluctuations in heart rate, as shown in the third column. Furthermore, these animals varied in their reaction to the continuation of further experiments so that some variations in the control rates became apparent. These variations in rate were taken into account and the average rates finally

measured showed the absence of cardiac acceleration except for the one experiment which produced an increased heart rate. In this experiment, the heart rate during the control period was rapid, and during distention the dog became excited, displayed tachypnea and dyspnea, and the heart rate showed an increase of 27 beats/min. Thus, while a cardiac acceleration was present, it differed from that following increase in venous return by having respiratory and affective concomitants which are absent when the venous return is increased.

CONCLUSIONS

Our experience reveals the presence of reflexogenic end-organs in the root of the superior vena cava, which in the chloralosanized animal can produce tachycardia, bradycardia and ectopic rhythms, but has apparently no effect in the unanesthetized animal with the exception of the solitary occurrence of tachycardia as part of the picture of dyspnea, tachypnea and excitement.

There is, therefore, no clear evidence to indicate that the region at the root of the superior vena cava is implicated in the cardiac acceleration following increase of the venous return. The location at which the Bainbridge reflex is initiated still remains to be determined.

We are indebted to several members of the department for technical assistance, especially to Mr. R. Asher.

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THE ORIGIN OF RENIN-ACTIVATOR

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Purified renin alone is not a vasopressor substance. This was shown by testing the various fractions leading to the purification of renin in the process of Helmer and Page (1) in both intact animals and isolated rabbit's ears perfused with Ringer's solution. As purification progressed it was observed that greater pressor responses occurred in intact animals and weaker vasoconstriction in perfused ears. From these experiments came the conclusion that during the chemical manipulations incident to purification of renin some substance had been lost requisite to its pressor action. This substance proved to be contained in blood plasma, for its addition, with a suitable period allowed for reaction, restored full activity. Fractionation of plasma showed the substance to be contained in the pseudoglobulins. It is heat labile, precipitated by ammonium sulfate and does not pass an ultra-filter. It has, therefore, the characteristics of a protein.

The logical conclusion from these experiments is that the pressor action of renin is due to some substance liberated by the interaction of renin and the pseudoglobulin fraction of blood. We have isolated and crystallized the reaction product and named it angiotonin. Independently Braun-Menendez, Fasciolo, Leloir and Muñoz (2) separated a substance having properties similar to those of angiotonin. They named it hypertensine.

When it was discovered that the pressor action of renin was an indirect one due to the formation of another substance, it seemed desirable to denote the members of the system with names as little suggestive of function as possible. We chose the name, "renin-activator," to connote merely that renin was inactive in its absence. It is true that the term "activator," has other meanings not necessarily applicable to the renin-angiotonin system and to that extent the term is undesirable. Braun-Menendez has preferred the term hypertensinogen, which implies that it is the substrate on which renin acts. Both groups of investigators have presented evidence that the reaction between renin and renin-activator is enzymatic in nature and both have suggested that the pseudoglobulin fraction was the substrate. But it must be emphasized that this has not been proved and that the published evidence does not justify the acceptance of the suggestion

that renin splits off angiotonin from a substrate variously called renin-activator or hypertensinogen. For these reasons, lacking a better term, we prefer to continue the use of the term "renin-activator," until such time as the mechanism of the reaction is fully understood.

As Page showed (3), one of the interesting characteristics of renin-activator is that it is present in blood in very limited amounts. Injection of renin quickly exhausts the store of renin-activator and leads to renin refractoriness or the so-called phenomenon of tachyphylaxis. Kohlstaedt, Page and Helmer (4) found renin-activator in the blood after hypophysectomy, adrenalectomy and nephrectomy. The most probable source is quite evidently the liver and it is the province of this paper to explore this possibility.

METHODS. Dogs of 12 to 15 kgm. body weight were used in all experiments. The action of the liver was either abolished by hepatectomy or reduced by administration by stomach tube of a mixture of equal parts of ethyl-alcohol and carbon tetrachloride.

At the initial operation an Eck-fistula was made using the technique of Fishback (5). From 2 days to 2 weeks later, the liver was removed, employing the technique of Markowitz, Yater and Burrows (6). The animals appeared to survive longer after hepatectomy if the period between operations was about 2 to 4 days rather than longer times. The mortality was very high when both operations were performed in one stage. After hepatectomy hourly injections of 0.25 gram of glucose per kilogram body weight were given.

Some of the animals lived as long as 14 hours, but many died in from 2 to 9 hours. At various intervals after hepatectomy the left common carotid artery was cannulated and blood pressure recorded from it. Angiotonin prepared by the method of Page and Helmer (7) and renin prepared by that of Helmer and Page (1) was injected into the femoral vein to ascertain their pressor responses. Samples of blood were taken into heparin solution, centrifuged and the plasma drawn off. The renin- and angiotonin-activator content of this plasma was determined by perfusion through a rabbit's ear after the addition of renin and angiotonin respectively, according to the method of Page (8). Hypertension was produced by the silk perinephritis method (9) or by silver clips applied to the renal artery sufficiently tightly to produce a distinct thrill just distal to the point of application.

RESULTS. Amounts of angiotonin and renin were administered for test purposes which gave a rise of arterial pressure of from 30 to 50 mm. Hg for renin and 15 to 25 mm. Hg for angiotonin in normal dogs. Often as short a time as 2 to 3 hours after hepatectomy, the pressor response to renin was completely abolished while that to angiotonin remained unaffected (table 1). Even after 8 to 14 hours the angiotonin response continued, long after renin elicited none.

TABLE 1

The effect of hepatectomy on the pressor responses to angiotonin and renin and the renin-activator content of plasma

DOG NO.	HOURS AFTER HEPATECTOMY	AMT. ANGIOTONIN	B.P. RISE	AMT. RENIN	B.P. RISE	AMT. PLASMA PERFUSED	RENIN ADDED TO PLASMA	PERFUSION OF RINGER'S SOLUTION THROUGH RABBIT'S EAR	
								Reduction of flow: time	Reduction of flow
		cc.	*mm. Hg*	*cc.*	*mm. Hg*	*cc.*		*minutes*	*per cent*
						0.2	0	1¼	53
1	0	0.2	14	0.3	56	0.2	+	4	87
						0.2	0	2¼	65
	4½	0.2	22	0.3	12	0.2	+	4¼	88
						0.2	0	2½	57
	10½					0.2	+	1½	65
						0.1	0	½	8
2	6	0.2	20	0.3	0	0.1	+	1¾	44
						0.1	0	½	23
	8	0.2	8	0.3	0	0.1	+	1¾	42
						0.1	0	1	47
3	0					0.1	+	4¼	88
						0.1	0	¾	21
	6	0.2	8	0.3	0	0.1	+	½	16
						0.1	0	1	25
	8	0.2	16	0.3	0	0.1	+	1	26
						0.1	0	¾	26
	9	0.2	10	0.3	0	0.1	+	1¼	31
						0.2	0	¼	7
4	0					0.2	+	5¼	47
						0.2	0	½	16
	1					0.2	+	3½	35
						0.2	0	¼	7
	2½	0.2	8	0.3	0	0.2	+	1½	12
						0.2	0	¼	14
	4	0.2	6	0.3	0	0.2	+	¼	4
						0.2	0	½	14
5	6	0.4	12	0.3	0	0.2	+	¾	20
						0.1	0	1¼	43
6	0					0.1	+	3¼	72
						0.1	0	½	8
	6	0.4	12	0.2	0	0.1	+	½	21
						0.1	0	¼	21
	8					0.1	+	½	21

TABLE 1—*Concluded*

DOG NO.	HOURS AFTER HEPATECTOMY	AMT. ANGIOTONIN	B.P. RISE	AMT. RENIN	B.P. RISE	AMT. PLASMA PERFUSED	RENIN ADDED TO PLASMA	PERFUSION OF RINGER'S SOLUTION THROUGH RABBIT'S EAR	
								Reduction of flow: Time	Reduction of flow
		cc.	*mm. Hg*	*cc.*	*mm. Hg*	*cc.*		*minutes*	*per cent*
						0.1	0	$1\frac{1}{4}$	47
7	6	0.2	10			0.1	+	1	22
8	2	0.4	12	0.3	0				
9	6	0.4	12	0.2	0				
						0.1	0	1	26
10	6					0.1	+	1	22
						0.1	0	$1\frac{1}{4}$	43
	8					0.1	+	$1\frac{3}{4}$	49
						0.2	0	$\frac{1}{2}$	10
11	0	0.3	18			0.2	+	$3\frac{1}{4}$	48
							0	$\frac{1}{2}$	10
	$3\frac{1}{2}$	0.3	16	0.3	2	0.2	+	$1\frac{1}{4}$	30
							0	$\frac{1}{2}$	10
	6	0.3	16	0.3	0		+	$1\frac{1}{2}$	21

Hepatectomy markedly and quickly reduced the renin-activator content of plasma. Its loss and the reduction or loss of the pressor action of renin paralleled one another. As an example, the plasma of one dog before operation caused, after the addition of renin, vasoconstriction in the rabbit's ear of 88 per cent of the initial value, lasting $4\frac{1}{4}$ minutes. Six hours later, when the pressor response of the intact animal had been lost, addition of renin to the plasma and perfusion through the ear caused only 16 per cent vasoconstriction, lasting $\frac{1}{2}$ minute. Clearly the operation of hepatectomy removed the chief source of renin-activator. Since angiotonin-activator (10) and the pressor response to angiotonin appear unaffected by hepatectomy (table 2, typical examples from 5 experiments) it is assumed that the liver is not the source of angiotonin-activator.

Hepatectomy was performed in seven hypertensive dogs. The striking result of this series of experiments was that death occurred within such a short time after operation. Normal dogs after hepatectomy usually survived from 6 to 11 hours after the operation, but the range in hypertensive dogs was from 1 to 4 hours. Loss of the pressor response to renin also occurred early—usually within an hour.

It was thought possible to produce a state similar to, but less severe than, that resulting from hepatectomy by injuring the liver by chemicals. Carbon tetrachloride-alcohol mixtures proved satisfactory though the varying amounts tolerated by different animals made uniformity in the experiments impossible. In normal animals administration of the mixture had no definite effect on arterial blood pressure until the animal became

TABLE 2

Effect of hepatectomy on renin- and angiotonin-activator content of plasma

CONDITIONS OF EXPERIMENT	RENIN ADDED			ANGIOTONIN ADDED		
	Plasma	Reduction of flow: time	Reduction of flow	Plasma	Reduction of flow: time	Reduction of flow
First experiment						
	cc.	*minutes*	*per cent*	*cc.*	*minutes*	*per cent*
Pre-operative	0.4	4	72	0.2	2½	61
10 hours post-operative	0.4	½	20	0.2	1	34
13 hours post-operative	0.4	½	17	0.2	1	30
Pre-operative				0.2	2½	56
13 hours post-operative				0.2	1	29
Second ear						
Pre-operative	0.4	4	65	0.2	3½	59
10 hours post-operative	0.4	½	15	0.2	2½	31
13 hours post-operative	0.4	½	17	0.2	2½	34
Second experiment						
Pre-operative	0.1	2½	68	0.1	4	84
6 hours post-operative	0.1	½	20	0.1	2½	57
8 hours post-operative	0.1	¼	12	0.1	2	41
Pre-operative	0.2	3½	69	0.1	3½	92
8 hours post-operative	0.2	¼	10			

very sick (table 3, examples taken from 6 similar experiments). The response to angiotonin was unaffected but that to renin was gradually lost. Again there was parallelism between the loss of renin-activator from the plasma and lack of response to renin. Equally true was that angiotonin-activator was unaffected.

The alcohol-carbon tetrachloride mixture was administered to 5 dogs with hypertension due to silk perinephritis. After several days to a week, arterial pressure usually fell and the renin-activator content of the plasma was reduced (table 4, examples from 5 experiments).

TABLE 3

The effect of administration of equal amounts of carbon tetrachloride and alcohol on the activator content of femoral blood and the responsiveness of normal dogs to injection of angiotonin and renin

DATE	B.P., MEAN	AMOUNT OF CCl_4-ALCOHOL ADMINISTERED	RENIN ADDED TO PLASMA			ANGIOTONIN ADDED TO PLASMA		RESPONSE OF DOG TO THE INJECTION OF				BILIRUBIN
				Rabbit's Ear Perfusion				Angiotonin		Renin		
			Amount plasma	Reduction of flow: time	Reduction of flow	Reduction of flow: time	Reduction of flow	Amount	B.P. rise	Amount	B.P. rise	
					Dog 15							
	mm. Hg	*cc./kgm.*	*cc.*	*minutes*	*per cent*	*minutes*	*per cent*	*cc.*	*mm. Hg*	*cc.*	*mm. Hg*	*mgm. per 100 cc.*
4/10/40	136		0.2	2½	68	2	42	0.5	16	0.1	10	
4/11/40			0.2	3	70	2½	47					
4/12/40	100		0.2	2	71	2½	58					
4/15/40	104		0.2	2	58	3	41					
4/17/40	102		0.2	1¼	47	2	46					
4/18/40	92							0.5	14	0.1	6	3.3
								1.0	20	0.3	12	
4/19/40			0.2	2	57	2	48					
4/22/40			0.2	2¼	61	2	39					
4/24/40	92		0.2	2	49	2	40					
4/26/40	100		0.2	1½	58	1½	44					
4/30/40	100	4.0	0.2	1½	56	1¼	46					
5/ 1/40		4.0	0.2	1½	49	2	53					
5/ 2/40	96	4.0	0.2	1¼	58	2	47					
5/ 3/40		4.0	0.2	1¼	60	2¼	45					4.3
5/ 6/40	94	4.0	0.2	¼	12	2	53					
			0.8	½	33							
			0.2	¼	12			0.5	12	0.1	0	
5/ 7/40			1.0	¼	21			1.0	18	0.3	4	5.5
					Dog 16							
4/12/40	108	4.0	0.2	3	70	2	54	1.0	20	0.3	26	
4/15/40	108	4.0	0.2	2¼	62	1½	40					
4/17/40	98	4.0	0.2	1	48	2¼	42	1.0	18	0.3	16	2.1
4/18/40			0.2	½	30	1	44					
4/20/40			0.2	0	0							
4/22/40	96		0.2	0	0	1	50					
4/23/40*	84		0.2	0	0	1	56	1.0	14	0.3	0	3.6
					Dog 17							
3/31/40	132	0	0.2	2	61	2½	40					
4/ 1/40	138	4	0.2	2	68	2¼	42					
4/ 2/40	148	2	0.2	2¼	61	1½	54					
4/ 3/40	138	4	0.2	2½	65	2	50					
4/ 4/40	150	8										
4/ 5/40	130	8	0.2	1	47	1½	51					
4/ 6/40		0	0.2	¼	31	1	40					
4/ 7/40	134	0	0.2	1	38							3.0
4/ 9/40	114	0										
4/ 9/40*												

* Dog died.

TABLE 4

The effect of administration of carbon tetrachloride plus alcohol to hypertensive dogs on the blood pressure and vasoconstrictor response to renin

DATE	B.P. MEAN	AMT. OF CCl_4-ALCOHOL ADMINISTERED	RENIN ADDED TO PLASMA		
			Amt. plasma	Reduction in flow: time	Reduction in flow
		No. 12			
	mm. Hg	*cc. per kgm.*	*cc.*	*minutes*	*per cent*
5/ 1/40	192	0			
5/ 7/40	186	0			
5/ 8/40	182	2	0.2	4½	70
5/ 9/40	146	2	0.2	2¼	79
5/10/40	140	2	0.2	½	34
5/11/40	120				
		No. 13			
6/18/40	220	0			
6/19/40	190	0			
6/20/40	186	2	0.2	1½	67
6/21/40	190	2	0.2	1	29
6/22/40	196	2			
6/24/40	200	2			
6/25/40	180	2	0.2	¼	16
		No. 14			
7/ 2/40	218	2	0.2	2¼	51
7/ 5/40	184	2	0.2	1¼	30
7/ 8/40	170		0.2	1½	46
7/ 9/40		1	0.2	¾	40
7/10/40			0.2	¼	12
7/11/40	200	2			
7/12/40	156	1	0.2	¼	24
7/15/40	178	1	0.2	¾	48
7/16/40	138	1			
7/23/40	112		0.2	1¾	49
8/ 2/40	138		0.2	2¼	60
8/12/40	228	1	0.2	2	28
8/15/40	194	1	0.2	2	59
8/16/40	174	1	0.2	1¾	65
8/19/40	190	1	0.2	1½	64
8/26/40	170	1	0.2		
8/28/40	193	1			
8/30/40	212	1.5			
9/ 3/40	184	1.5			
9/ 4/40		1.5			
9/ 5/40		1.5			
9/ 6/40	200	1.5			
9/ 9/40	198	1.5			
9/14/40	198	1.5			
9/16/40	174	1.5	0.2	2½	46
9/19/40	130	1.5			

DISCUSSION. Several hours after hepatectomy in dogs the renin-activator content of plasma is significantly reduced and in most cases almost disappears and simultaneously, the pressor response to injected renin is decreased or abolished. On the other hand, angiotonin-activator is not changed by the operation and the pressor response to angiotonin continues unimpaired. The most reasonable explanation for these observations is that extirpation of the liver removes the source of renin-activator and, since it is necessary for the formation of angiotonin, no pressor response occurs when renin is injected into the circulation. That the animal is capable of response to other pressor agents is shown by the fact that angiotonin continues to cause a rise of arterial pressure when renin does not.

The experiments in which carbon tetrachloride-alcohol mixtures were used to damage the liver and so reduce the renin-activator were partially successful. The difficulty with such experiments is that the tolerance for carbon tetrachloride and alcohol in different dogs is extraordinarily variable. Some die after three or four doses of 4 cc. per kilogram body weight while others will tolerate 10 to 20 times as much.

The arterial pressure and renin-activator in hypertensive dogs tends to decrease when sufficient amounts of carbon tetrachloride-alcohol mixture are given. This might be interpreted as indicating a direct relationship between the pressure change and renin-activator change. Since carbon tetrachloride and alcohol are toxic to other parts of the body, these experiments can only be used to support those in which the liver is actually removed.

SUMMARY. 1. Removal of the liver in dogs causes the renin-activator in the plasma to be reduced or disappear. Simultaneously the pressor response to injected renin is reduced or disappears.

2. Hepatectomy has no marked influence on the angiotonin-activator content of the blood or the pressor response to injected angiotonin.

3. Administration of mixtures of carbon tetrachloride and ethyl alcohol by mouth to normal dogs causes both the renin-activator content of the plasma and the pressor response to injected renin to be reduced.

4. The arterial blood pressure and renin-activator content of plasma of hypertensive dogs is reduced by administration of carbon tetrachloride-alcohol mixtures. The tolerance of different dogs to these toxic substances is extraordinarily variable.

5. Hepatectomy appeared to cause death in hypertensive animals more quickly than in normal ones, and renin-activator tended to disappear more rapidly.

CONCLUSION

The liver is the chief source of renin-activator and its removal or damage by toxic substances in normal and hypertensive dogs reduces the renin-

activator content of plasma and hence the pressor response to injected renin. The pressor response to angiotonin and the angiotonin-activator of blood remains unaffected by these procedures.

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STUDIES ON NORMAL AND DEPANCREATIZED DOMESTIC DUCKS[1]

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It has been known for some time that all species do not respond in the same manner to the removal of the pancreas. Of particular interest in this regard is the observation first made by Minkowski (1) and subsequently confirmed by others, that the depancreatized domestic fowl does not develop the hyperglycemia and glycosuria that occur in similarly treated dogs or cats. The fact that removal of the pancreas from the chicken or duck leaves no other source of insulin (2) but apparently permits the animal to utilize carbohydrate in a normal manner, has raised numerous speculations. It is possible to deduce from this that following pancreatectomy in birds there is some change in the mechanism of carbohydrate metabolism which enables them to dispense with insulin. On the other hand, the lack of metabolic changes may be due to some inherent deficiency in the activity of the pituitary or adrenal glands of the fowl (3). However, attempts to establish the character of the metabolic and glandular changes consequent to pancreatectomy in the fowl has thus far been unsuccessful.

Although it has been shown that in spite of the total removal of the pancreas from chickens and ducks the liver can store glycogen (3), the respiratory quotient is elevated by feeding glucose (4), the response to the injection of exogenous insulin is normal (3), few studies have been performed with relation to the metabolism of fat in the depancreatized duck. In an attempt to obtain further information concerning this interesting phenomenon, we studied some aspects of carbohydrate and fat metabolism of both normal and depancreatized domestic ducks.

EXPERIMENTAL. One hundred and thirty-five white domestic ducks (bred by the Camp Creek Duck Farms), weighing initially from 4 to 5 pounds were used in this study. For operation, animals were anesthetized

[1] Presented in part before the American Physiological Society, March 1940, New Orleans, La.

[2] Leo Lehman Fellow.

with an intraperitoneal injection of from 60 to 75 mgm. of sodium amytal per kilo of body weight. By careful dissection the whole pancreas was removed; completeness of removal was verified by a subsequent post mortem examination. Blood sugar was determined by the Somogyi modification of the Shaffer-Hartman method, and the total acetone bodies by a modification of Barnes' procedure (5). Samples of blood were drawn from the wing vein before the operation and at daily intervals thereafter. The various experiments that were performed with both normal and depancreatized ducks are discussed below.

Blood sugar and body weight changes. Two procedures were employed in studying the changes consequent to pancreatectomy in the duck. Immediately following the operation, one set of animals was permitted to eat freely of a "duck grower" diet,[3] whereas another set of depancreatized ducks was deprived of food for about 30 days.

In confirmation of the observations of others, it was noted that extirpation of the pancreas resulted in a relatively insignificant change in the blood sugar level. Even if a slight increase in blood sugar occurred immediately after the operation, a return to normal was observed to take place within one week.

Immediately after the pancreatectomy, there was a gradual but definite loss in body weight. Thus, the average change in weight of the depancreatized-fed animals was a drop of 16 per cent in 9 days and 38 per cent in 30 days; of the depancreatized-fasted animals, a drop of 22 per cent in 9 days and 48 per cent in 30 days; of the normal-fasted ducks, a drop of 17 per cent in 9 days and 42 per cent in 30 days. Comparison of these data reveals that there was very little difference between the loss of weight of the normal-fasted, depancreatized-fed, and depancreatized-fasted groups of animals.

In order to determine whether the loss in weight was due to some specific metabolic change associated with the loss of insulin or to the loss of the external secretion of the pancreas, a series of depancreatized animals was given a diet containing 74 parts "duck grower," 24 parts raw pancreas, and 2 parts pancreatin.[4] It was observed that with the ingestion of pancreas and pancreatin, the loss of weight was retarded partially or even completely in the depancreatized ducks. This also occurred when pancreatin alone was added to the duck diet. Thus, a depancreatized duck which weighed 1.5 kgm. was placed on a diet containing pancreatin, and in twelve days the weight rose to 2.01 kgm. In spite of the fact that the pancreatin retarded the loss of weight, no effect on the blood sugar level was noted.

[3] Purchased from Camp Creek Duck Farms, Monticello, Illinois.

[4] We are indebted to Dr. David Klein of the Wilson Laboratories for generous supplies of pancreatin.

This suggests that insulin insufficiency is not the factor responsible for the loss of weight.

Sugar tolerance curves. Three types of studies were performed utilizing the sugar tolerance curve. Tests were performed on fed-depancreatized ducks (18 hrs. after withdrawal of food) within two to three days after pancreatectomy and again in the second or third week after pancreatectomy. In all instances, 1.75 gram of glucose per kg. was injected intravenously (as a 30 per cent solution) and blood samples drawn at various intervals thereafter. No significant differences between the blood sugar curves of the normal and depancreatized ducks could be demonstrated (fig. 1, curves *1* and *3*).

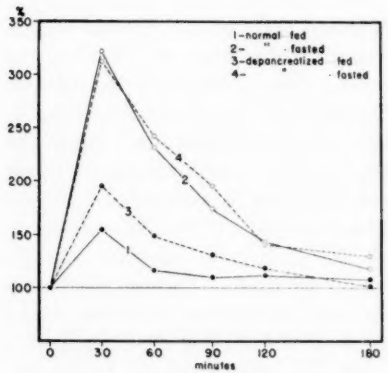

Fig. 1

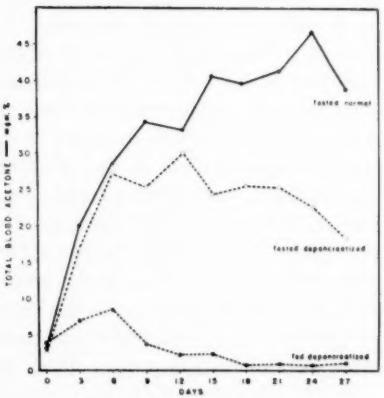

Fig. 2

Fig. 1. Sugar tolerance curves of fed and fasted normal and depancreatized ducks.
Fig. 2. The influence of pancreatectomy on the accumulation of acetone bodies in the blood of the duck.

Since it is known that the repeated administration of glucose will result in an exaggerated disturbance of the blood sugar curve of depancreatized dogs or cats, this phenomenon was likewise studied in depancreatized ducks. One and seventy-five hundredths gram of glucose per kg. was administered intravenously to fed depancreatized ducks at hourly intervals and blood samples drawn just before each injection. No significant differences between the curves of the normal and of the depancreatized ducks could be demonstrated.

A comparison of the sugar tolerance curve of fasted and fed normal and depancreatized ducks was made. It was observed that the normal duck, after a period of fasting of from 10 to 20 days, developed a typical diabetic response. A similar response was observed in the depancreatized ducks

even though normal curves had been obtained previously in the same ducks when they were not fasting (fig. 1, curves *2* and *4*).

Ketogenesis. Although a number of investigators have studied various aspects of the carbohydrate metabolism of the depancreatized duck, we are not aware of similar studies with reference to fat metabolism. It is now known that the acetone bodies, β-hydroxybutyric and acetoacetic acid are normal intermediaries in fat oxidation, and that these substances are utilized quite freely by the muscles of mammals (6). Furthermore, it is known that for the dog, rat and rabbit the rate of acetone body utilization is dependent upon the concentration of these bodies in the tissues, and that up to a certain level the rate of utilization parallels the blood concentration (7). Hence, by studying the ketonemia of depancreatized ducks, it may be possible to estimate not only the rate of acetone body formation, but also to obtain some index of acetone body utilization. In this way a criterion of the rate of fat metabolism may be obtained. It was observed that the fed, depancreatized duck showed a slight ketonemia immediately after operation which dropped to an insignificant level within one week. The normal-fed duck on the other hand showed no ketonemia. When the normal duck was fasted, a rapid accumulation of acetone bodies in the blood stream occurred. The fasted, depancreatized duck likewise showed a rapid accumulation of acetone bodies in the blood up to about the sixth day of the fast when the acetone body level became relatively constant (fig. 2). Subsequently, a decrease in the blood level occurred. This observation is of interest especially when one compares the ketonemia of fasting ducks with that of fasting dogs. Thus, the fasted normal dog develops a very slight ketonemia after 9 days of fast. On the other hand, the fasted, depancreatized dog develops a more severe ketonemia after a similar interval of time. The impression gained from these studies is that whereas pancreatectomy increases the susceptibility to fasting ketosis in the dog, the reverse is the rule with the duck (fig. 3).

In order to accelerate the rate of fat catabolism, the influence of phlorhizin administration in normal and depancreatized ducks was studied. It is a well known phenomenon that the administration of phlorhizin to the depancreatized dog results in an acceleration of the rate of acetone body accumulation in the blood to a much more marked degree than can be observed in the normal dog. However, in the case of the duck, the daily subcutaneous administration of ½ gram of phlorhizin suspended in oil resulted in a much greater accumulation of acetone bodies in the blood of the normal duck than in that of the depancreatized duck (fig. 4). Blood levels as high as 170 mgm. per cent were observed in some phlorhizinized normal ducks. Of interest is the fact that after approximately 15 days of phlorhizin administration the depancreatized duck shows a drop in the blood acetone body level, whereas the normal duck shows a continued

accumulation of these bodies. Blood sugar determinations made simultaneously with the acetone body determinations revealed an insignificant decrease in the blood sugar level, and nitrogen excretion data likewise showed insignificant changes from the control.

Hepatectomy. In order to determine the influence of hepatectomy, the livers from the normal and depancreatized ducks were removed and the rate at which the blood sugar fell was determined. No significant difference was observed between the drop in the blood sugar of normal and of depancreatized ducks.

Influence of various hormones. In view of the suggestion that the anterior pituitary may function inefficiently in the duck insofar as carbohy-

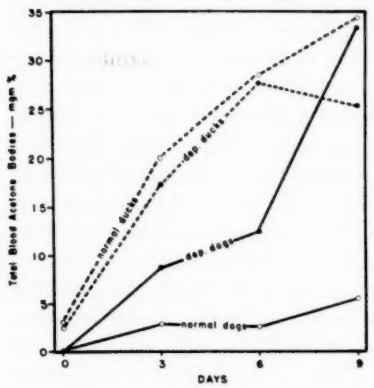

Fig. 3

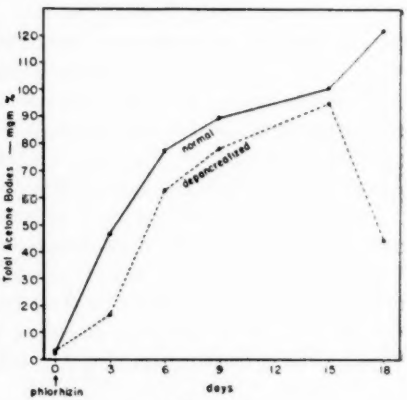

Fig. 4

Fig. 3. Comparison of the ketonemia of fasted ducks and dogs.

Fig. 4. Illustrating the influence of the daily subcutaneous injection of phlorhizin on the level of total acetone bodies in the blood of normal and depancreatized ducks.

drate metabolism is concerned, it became of interest to study the influence of various extracts. Fresh beef glands were extracted according to the procedure outlined by Young (8) and intraperitoneal injections of the equivalent of from 1 to 5 grams of whole anterior pituitary glands were given daily. No effect of these injections on the blood sugar of the depancreatized duck was observed. This response differs markedly from that observed with mammals, since in the latter the administration of such extracts after complete or partial pancreatectomy results in a marked exaggeration of the diabetic syndrome.

Since it has been observed by Zondek and Marx (9) and by Lorenz, Chaikoff and Entenman (10) that estrogenic materials will raise the blood fats of chickens, it was considered of interest to study the influence of the

administration of estrin on the blood sugar level and on the rate of acetone body accumulation in the blood. No significant effect was noted.

Discussion. Our data do not give any clue to the mechanism whereby the depancreatized duck maintains an apparently normal state of carbohydrate metabolism. The loss of weight consequent to pancreatectomy can be attributed to a loss of the external secretion as is suggested by the fact that the administration of pancreatin or of raw pancreas permits the depancreatized duck to maintain its weight or even to gain weight. The fact that diabetic sugar tolerance curves occur in both normal and depancreatized ducks on fasting but not when fed suggests that the delay in the removal of exogenous sugar from the blood stream is independent of the presence of the pancreas.

Of interest is the observation that the normal-fasted duck develops a more marked ketonemia than do similarly treated dogs. The fact that with feeding, the depancreatized duck does not develop a significant ketonemia is in accord with the observations on mammals. The only metabolic disturbance that we have observed that may be attributed to the loss of the pancreas is the somewhat slower rate of acetone body accumulation in the depancreatized duck as compared with the normal duck. Furthermore, the acetone body level is not maintained at as high a level for as long an interval in the depancreatized duck as in the normal duck. This is also true for the phlorhizinized animals. These data suggest that some disturbance either in the transport of fat from the depots or in the oxidation of fat in the liver ensues in consequence of pancreatic removal. This need not be due necessarily to a loss of insulin, for the recent studies of Montgomery, Entenman, Chaikoff and Nelson (11) indicate the presence of an essential factor for fat metabolism in the external secretion of the pancreas.

It is possible that a fatty liver may be a factor in the prevention of hyperglycemia, a phenomenon which is known to occur in the depancreatized dog. However, analysis of the livers of many depancreatized ducks revealed concentrations of glycogen and fat similar to those observed in normal ducks. The studies with saline extracts of the anterior pituitary and with estrin suggests that an inadequacy of the anterior pituitary is not the factor responsible for the state of the depancreatized duck.

In view of the fact that most herbivorous animals develop a relatively mild degree of diabetes consequent to pancreatectomy, it occurred to us that the nutritional habits of the species may be an important factor. Hence, an attempt was made to convert the domestic duck, which is a herbivorous animal, to a carnivorous one. For that purpose one day old ducks were started on a diet consisting of raw ground beef and this diet was continued until the animals were approximately one year of age. The pancreas of such an animal is not normal, in that it is much smaller in

size and quite fibrotic. Removal of the pancreas from such animals resulted, in many instances, in a marked hyperglycemia to levels as high as 500 mgm. per cent on the first, second and sometimes third day after the operation, at which time the animals either died or the blood sugar returned to normal. The administration of insulin immediately after the operation did not prolong their lives. Although suggestive, this study does not indicate that the meat fed duck is essentially more susceptible to the development of diabetes than is the herbivorous duck.

SUMMARY AND CONCLUSIONS

Pancreatectomy of the duck does not result in any significant disturbance in the blood sugar level, the sugar tolerance curve, or in the response to the repeated injection of sugar.

The ingestion of pancreatin and raw pancreas inhibits the loss of weight consequent to pancreatectomy.

The sugar tolerance curves of fasted ducks are equally prolonged in both normal and depancreatized ducks, suggesting that a lack of insulin is not responsible for the diabetic curve of fasting.

Fasting induces a greater ketosis in the normal duck than in the depancreatized duck.

Phlorhizin administration results in a more marked ketonemia in normal than in depancreatized ducks.

The ketonemia of the fasted-normal duck is greater than that of similarly treated dogs.

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CERTAIN EFFECTS OF DESOXYCORTICOSTERONE

The Development of "Diabetes Insipidus" and the Replacement of Muscle Potassium by Sodium in Normal Dogs[1]

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It has been shown that a syndrome resembling diabetes insipidus can be produced in normal dogs by repeated injections of desoxycorticosterone acetate (1, 2). With the administration of desoxycorticosterone there is a progressive increase in fluid intake and urine volume, a fall in urine specific gravity, a slight but consistent rise in serum sodium concentration and a fall in the serum potassium. The "diabetes insipidus" becomes quite marked when salt is added to the diet; and when the syndrome is well established, attacks of profound muscular weakness make their appearance. These periodic attacks of muscular weakness or paralysis can be alleviated by the administration of potassium salts, and although it is difficult to detect a significant disturbance of sodium or potassium balance during the gradual development of the syndrome (1, 2), it has been found in the present study that the skeletal muscles of animals dying in paralysis contain large amounts of sodium and less than normal amounts of potassium. Similar changes in the muscle electrolytes of rats given desoxycorticosterone have been observed by Darrow (3). These observations suggest a possible exchange between extracellular sodium and intracellular potassium. Because an exchange of this type is contrary to accepted doctrine, further investigation of the problem seemed warranted.

Methods. Six normal dogs, four male and two female, were placed upon a diet of commercial dog food[2] and their weights and fluid intakes measured during a three week period of preliminary observation. At various times during the study samples of blood for serum electrolyte analysis were taken and specimens of skeletal muscle for chemical analysis and histological study were obtained under nembutal anesthesia from the neck and leg muscles of each animal.

[1] Supported in part by a grant from the Carnegie Corporation.

[2] Arcadia Milling Co., Chicago, Ill.

The results of the histological studies will be reported at another time.

For the chemical analyses approximately 10 grams of wet muscle were weighed in a tightly stoppered weighing bottle of known weight, minced and allowed to dry to constant weight in an electric oven at 70 to 90° centigrade. The dried muscle was then transferred quantitatively to a mortar and thoroughly ground and mixed. The resulting powder was placed in a weighing bottle and all samples for analysis were weighed out from it by difference.

In the determination of sodium and potassium, duplicate 0.4 gram samples of dried muscle powder were placed in platinum dishes and ashed in an electric muffle at 470 degrees centigrade. The ash was transferred to 10 cc. volumetric flasks containing 1 drop of phenolphthalein using one drop of concentrated hydrochloric acid in the first portion of water to dissolve the ash. After the flasks were made up to volume, a small amount of calcium oxide was added, enough to make the solution alkaline, and the flasks were shaken and allowed to stand over night. The phosphate-free solution of ash was then filtered through a 5.5 cm. no. 42 Whatman filter paper into a small Erlenmeyer flask. One-half cubic centimeter of filtrate was transferred to a 20 cc. volumetric flask, neutralized with 1 drop of glacial acetic acid and made up to volume. Two cubic centimeter aliquots were taken for potassium analysis by the cobaltinitrite method (4). For the sodium determinations 7 or 8 cc. aliquots were transferred to 30 cc. beakers, made acid with one drop of concentrated hydrochloric acid and carefully evaporated in the oven to a final volume of 1 or 2 cc. Sodium was then precipitated by the uranyl zinc acetate method (5).

For the determination of chloride, duplicate 0.3 gram samples of dried powder were transferred to 50 cc. round-bottom centrifuge tubes. Three cubic centimeters of 0.05 normal silver nitrate in concentrated nitric acid and 3 cc. of 8 per cent potassium permanganate were added and the chlorides determined by the procedure of Wilson and Ball (6).

For the determination of nitrogen, duplicate 0.03 gram samples of dried powder were weighed out and analyzed by the micro-Kjeldahl technique (7). The weighed sample was digested with 2 cc. of concentrated sulphuric acid copper sulphate mixture, a glass bead and approximately 0.5 gram of potassium sulphate until clear and colorless. The digest was then transferred quantitatively to a 100 cc. volumetric flask and made up to volume. Fifteen cubic centimeter aliquots were distilled, after the addition of 10 cc. of 50 per cent sodium hydroxide, into saturated boric acid and the ammonia titrated with N/70 normal hydrochloric acid using methyl red as the indicator.

For the determination of fat content approximately 0.5 gram samples of dried powder were placed in weighed centrifuge tubes. Five cubic centimeters of ethyl ether were added and the mixture was thoroughly stirred

with a fine stirring rod. The rod was rinsed with 3 cc. more of ether and the mixture was allowed to stand over night. It was then centrifuged and the supernatant carefully decanted. Five cubic centimeters of petroleum ether were added and the mixture again allowed to stand over night, centrifuged, and again decanted. The remaining material was allowed to dry to constant weight by evaporation. The total weight lost by the sample was considered to represent its fat content.

For the chemical analysis of serum slight modifications of the usual standard methods were used (4, 5, 6, 7).

Following the control observations the animals were divided into two groups of three each. The diet was maintained as usual in both groups but one group was given a solution containing 0.15 per cent potassium chloride to drink while the other group received ordinary tap water. All the animals were then given daily subcutaneous injections of 25 mgm. of desoxycorticosterone acetate dissolved in peanut oil.[3]

Results. As a result of the treatment with hormone there was a slight rise in the serum sodium concentration, about 4 or 5 milliequivalents per liter and a fall in serum potassium concentration, about 1 to 1.5 milliequivalents per liter (table 1). The rise in serum sodium concentration was approximately equal in both groups of animals but the fall in serum potassium concentration appeared less in the animals receiving potassium chloride in the drinking water (table 1). Accompanying these changes in serum electrolyte concentrations a rather marked increase in fluid intake occurred, both in the animals receiving potassium chloride and in the animals not receiving potassium chloride. The intake of fluid gradually rose from a daily average of approximately 500 mil. at the start of the experiment to about 3500 mil. at the end of four weeks' treatment with desoxycorticosterone, quite independent of whether or not the animals received potassium chloride.

During the fourth and fifth weeks of hormone administration a striking difference between the animals receiving potassium chloride and the animals not receiving potassium chloride became apparent. The animals that did not receive potassium chloride became at first intermittently and then severely paralyzed and died. The animals that received potassium chloride remained in good health and had no symptoms of paralysis. When the administration of potassium chloride was stopped, however, paralysis appeared in about four days and was severe in ten days. In other words, there was unequivocal evidence that administration of potassium chloride prevented the occurrence of attacks of paralysis but had no effect upon the development of diabetes insipidus.

[3] Obtained through the courtesy of Roche-Organon, Inc., Nutley, N. J.

The results of the chemical analysis of skeletal muscle removed at various intervals during the experiment are presented in table 2. The paralysis-preventing action of potassium chloride appeared related to the maintenance of a normal electrolyte pattern in the muscle cells. Figure 1 shows the average amounts of sodium and potassium found in a kilogram of fresh dog muscle in the several circumstances of the experiment. In

TABLE 1

*Chemical analysis of serum of dogs before and after treatment with Doca**

DOG	TREATMENT	Na	K	Cl	PROTEIN	NPN	REMARKS
		m.eq./l.	*m.eq./l.*	*m.eq./l.*	*grams per cent*	*mgm. per cent*	
1	Untreated	146.9	3.9	107.0			
2	Untreated	146.0	4.0	103.8			
	1 month Doca alone	149.1	2.9	107.5	5.2	20	Paralyzed
3	Untreated	144.0	3.9	107.7			
	1 month Doca alone	150.9	2.9	102.0	6.9	22	Paralyzed
4	Untreated	147.6	4.7	107.0			
	1 month Doca and KCl	154.4	3.5	103.6	6.1	22	No weakness
	2 months Doca and KCl	150.1	3.5	109.1	6.5	19	No weakness
	8 days Doca alone		2.2				Slight weakness
5	Untreated	144.4	4.2	106.8			
	1 month Doca and KCl	150.0	2.8	105.6	6.5	22	No weakness
	2 months Doca and KCl	147.4	3.0	107.7	7.0	20	No weakness
	8 days Doca alone		3.1				No weakness
	14 days Doca alone	154.8	3.4	110.4	6.4	32	Paralyzed
6	Untreated	144.5	4.0	105.0			
	1 month Doca and KCl	150.0		108.2	6.3	16	No weakness
	2 months Doca and KCl	148.3	4.2	107.8	7.4	19	No weakness
	8 days Doca alone		2.6				Slight weakness
	14 days Doca alone	148.1	2.7	98.2	6.0	25	Paralyzed

* Desoxycorticosterone acetate.

the first column are represented the amounts of sodium and potassium found under the normal control conditions before any hormone was given. As is usual for dog muscle, there were about 35 m.eq. of sodium and 80 m.eq. of potassium per kilo of fresh muscle tissue. Similar normal values for sodium and potassium were found in muscle biopsies taken from dogs receiving potassium chloride and hormone. On the other hand the biopsies of muscles taken from paralyzed dogs showed markedly abnormal con-

centrations of sodium and potassium, averaging 70 m.eq. of sodium instead of the normal 35, 50 m.eq. of potassium instead of the normal 80.

Since the chloride, water and nitrogen content of muscle taken from

TABLE 2

*Chemical analysis of muscle of dogs before and after treatment with Doca**

DOG	TREATMENT	MUSCLE	SOLIDS† FAT-FREE	FAT IN FRESH MUSCLE	NITROGEN†	Na†	CALCULATED INTRACELLULAR Na†	K†	Cl†	REMARKS
			grams	*per cent*	*grams*	*m.eq.*	*m.eq.*	*m.eq.*	*m.eq.*	
1	Untreated	Leg	219	1.2	32.7	33.9	**3.4**	82.5	24.7	
	Untreated	Back	221	3.2	33.6	38.8	**6.6**	82.0	26.1	
	1 month Doca alone	Leg	225	1.9	34.6	55.6	**21.3**	54.0	26.6	Fatal paralysis‡
	1 month Doca alone	Neck	217	2.8	32.6	65.8	**24.0**	56.1	32.4	Fatal paralysis‡
2	Untreated	Leg	232	1.7	34.9	33.6	**4.2**	80.3	23.2	
	Untreated	Back	240	3.4	34.3	34.2	**7.5**	83.9	21.2	
	1 month Doca alone	Leg	208	0.6	30.9	61.8	**24.1**	46.9	30.1	Paralyzed
	6 weeks Doca alone	Neck	236	0.6	36.8	91.2	**51.0**	47.5	31.1	Fatal paralysis‡
3	Untreated	Leg	219	2.3	33.0	41.1	**9.1**	75.4	26.6	
	1 month Doca alone	Leg	231	1.8	34.1	49.9	**21.1**	65.9	21.8	Paralyzed
	1 month Doca alone	Neck	205	2.5	30.6	88.3	**43.3**	42.3	34.0	Paralyzed
	7 weeks Doca alone	Neck	247	1.1	36.0	83.0	**49.3**	53.7	26.1	Fatal paralysis‡
4	Untreated	Leg	229	0.9	35.0	26.7	**3.6**	91.0	18.6	
	1 month Doca and KCl	Leg	235	1.2	35.0	33.0	**8.0**	84.9	18.6	No weakness
	1 month Doca and KCl	Neck	228	1.3	34.9	39.2	**6.7**	84.6	24.2	No weakness
	2 months Doca and KCl	Leg	221	1.8	33.9	37.2	**11.5**	82.0	20.7	No weakness
	13 days Doca alone	Leg	194	1.4	29.4	116.0	**57.3**	31.2	45.4	Fatal paralysis‡
	13 days Doca alone	Neck	209	1.7	29.5	89.3	**34.1**	49.9	42.7	Fatal paralysis‡
5	Untreated	Leg	204	2.0	30.3	41.6	**6.8**	80.0	28.6	
	1 month Doca and KCl	Leg	220	2.0	33.1	34.8	**10.3**	83.2	19.2	No weakness
	1 month Doca and KCl	Neck	219	3.2	33.6	38.1	**7.3**	89.7	24.1	No weakness
	2 months Doca and KCl	Leg	214	3.0	32.6	37.9	**8.7**	89.3	23.7	No weakness
	2 weeks Doca alone	Leg	196	5.4	29.6	82.8	**34.2**	51.6	38.5	Paralyzed
	2 weeks Doca alone	Neck	214	2.9	32.9	52.4	**18.2**	72.9	27.1	Paralyzed
6	Untreated	Leg	223	0.7	33.3	33.3	**1.8**	76.4	25.4	
	Untreated	Back	226	1.0	31.2	34.4	**2.9**	74.8	25.4	
	1 month Doca and KCl	Leg	234	1.5	34.6	27.8	**6.2**	83.4	17.3	No weakness
	1 month Doca and KCl	Neck	210	1.8	31.8	43.1	**8.9**	79.4	27.4	No weakness
	2 months Doca and KCl	Leg	214	2.5	32.4	49.7	**15.0**	70.6	28.0	No weakness
	2 weeks Doca alone	Leg	198	1.1	30.3	79.8	**40.8**	45.4	28.5	Paralyzed
	2 weeks Doca alone	Neck	212	1.2	31.7	62.1	**29.7**	59.9	23.7	Paralyzed

* Desoxycorticosterone acetate.

† Calculated per kilogram of fat-free fresh muscle.

‡ Postmortem specimen.

paralyzed animals was essentially normal (fig. 2) there appeared no reason to suppose that the extreme changes in sodium and potassium concentrations were related to an alteration in either the volume or the composition of the extracellular fluid. Furthermore, calculation of the amount of

intracellular sodium present in paralyzed muscle[4] indicated that in animals developing paralysis approximately 30 per cent of the potassium of the skeletal muscle had been replaced by sodium (fig. 3). As shown in figure 3 the replacement of potassium by sodium appeared to be on an approximately 1 to 1 basis, an atom of sodium being gained by the muscle cell for each atom of potassium lost.

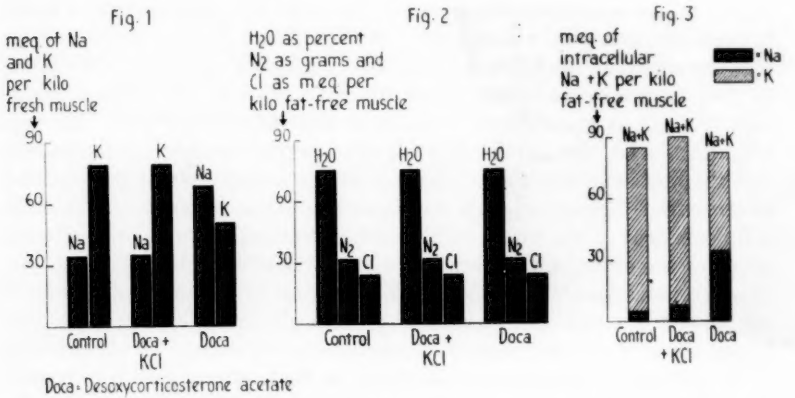

Fig. 1. The effect of desoxycorticosterone acetate upon the concentration of sodium and potassium in dog muscle.

Fig. 2. The effect of desoxycorticosterone acetate upon the water, nitrogen and chloride content of dog muscle.

Fig. 3. The effect of desoxycorticosterone acetate upon the concentration of intracellular sodium and potassium in dog muscle.

Discussion. From the experiments which have been described it seems possible to draw certain conclusions concerning the effect of desoxycorticosterone upon normal dogs. Administration of this hormone has been shown to cause a rise in serum sodium concentration and a fall in serum potassium concentration which, when continued long enough results in a striking

[4] Calculation:

Milliequivalents of sodium per kgm. fat-free muscle ..... Na_T
Milliequivalents of chloride per kgm. fat-free muscle ..... Cl_T
Milliequivalents of sodium per l. of serum ..... Na_S
Milliequivalents of chloride per l. of serum ..... Cl_S

Assuming that all muscle chloride is extracellular and using 0.95 as a correction factor for the Donnan effect between serum and extracellular fluid, the intracellular sodium may be calculated as:

$$Na_T - \left[Cl_T \left(\frac{Na_S \cdot 0.95}{\frac{Cl_S}{0.95}} \right) \right] = \text{milliequivalents of intracellar sodium per kgm. of fat-free muscle.}$$

replacement of intracellular potassium by sodium. In accordance with previous experience, these electrolyte changes have been shown to be associated with the appearance of "diabetes insipidus" which later is complicated by attacks of profound muscular weakness. By administering potassium chloride to animals receiving hormone it has been found possible to differentiate, to a certain extent, the mechanism of the "diabetes insipidus" from the mechanism underlying the attacks of muscular weakness. It has been found, for example, that ingestion of potassium chloride in adequate quantity will prevent the disturbance of muscle electrolytes and the muscular weakness without affecting either the diabetes insipidus or the elevated serum sodium concentration. As a result of this observation it seems permissible at the present time to associate the "diabetes insipidus" with the persistently elevated serum sodium concentration, and the muscular weakness entirely with the disturbance of intracellular electrolytes. Moreover, since the protective action of potassium chloride is independent of the serum sodium concentration, it seems preferable to speak of the effect of the hormone upon intracellular electrolytes as one in which potassium lost from the cells is *replaced* by sodium, rather than as one in which a high concentration of extracellular sodium in some way brings about a *displacement* of intracellular potassium. In other words in the present experiments it would appear that sodium goes into muscle cells only when potassium comes out. This view is consistent with the idea that desoxycorticosterone, by increasing the renal excretion of potassium, lowers the serum potassium concentration and shifts the equilibrium between cellular and extracellular potassium in the direction of an increased loss of potassium from the cell. In further support of this interpretation are the observations of Heppel (8). This worker showed that potassium deprivation during the growth of young rats resulted in partial replacement of intracellular muscle potassium by sodium. Thus it appears that when, by one means or another, the available supply of potassium is reduced, sodium replaces potassium lost from the muscle cell.

CONCLUSION

1. Normal dogs receiving daily injections of 25 mgm. of desoxycorticosterone acetate develop attacks of muscular weakness and a syndrome resembling diabetes insipidus.

2. The attacks of paralysis are associated with a partial replacement of the intracellular potassium of the skeletal muscles by sodium.

3. The paralysis and the disturbance of muscle electrolyte concentrations can be prevented by the administration of potassium chloride.

4. In the reported experiments the "diabetes insipidus" is consistently associated with an elevation of serum sodium.

5. Neither the diabetes insipidus nor the elevation of serum sodium is influenced by the administration of potassium chloride.

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ADDITIONAL EVIDENCE OF QUALITATIVE DIFFERENCES BETWEEN THE RESTING AND ACTIVITY OXYGEN CONSUMPTIONS OF FROG MUSCLE[1]

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In an earlier publication (Stannard, 1939a) it was shown that the resting oxygen consumption of frog muscle, while sensitive to cyanide, was not inhibited by sodium azide (NaN_3) except at very high concentrations and/or after long periods of exposure. On the other hand any increments in respiration above the resting level caused by electrical or chemical stimulation were specifically inhibited by azide. These data suggested that the resting and "activity" respirations of frog muscle were qualitatively distinct and that the cytochrome-cytochrome oxidase system functioned only to carry the extra oxygen consumption associated with electrical or chemical stimulation.

Whether or not the proposed qualitative separation of resting and "activity" respirations extends to other steps in the metabolic chain or involves only the cytochrome system seemed an important inherent question. With this problem in mind a study was made of the effects of several other types of inhibitors on the respiration of resting and caffeinized frog muscle. The results indicate that the two types of respiration probably are separable beyond the oxidase step. Thus none of the substances tested affected the resting respiration in the manner expected of a specific inhibitor while sodium arsenite and sodium fluoride effectively eliminated or prevented the extra oxygen consumption due to caffeine. Furthermore additional substances which may attack cytochrome oxidase (hydroxylamine and the "copper-inhibitors," diethyldithiocarbamate and potassium ethyl xanthate) brought about marked inhibition of the respiration of caffeinized muscle without decreasing appreciably the resting respiration. In fact the points of action of the inhibitors employed are sufficiently different to suggest, barring proof to the contrary, that the two respiratory systems may be completely independent.

It is felt that the data obtained combined with studies made in other

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laboratories (see discussion) strengthen materially the evidence that cells in activity (i.e., performing their specific function) may utilize enzymes or enzyme systems present but not operative in the "resting" state.

METHODS. Oxygen consumption was measured in Warburg manometers (vessel size approximately 15 ml.) or Fenn differential manometers when the rates of respiration to be measured were low or the amount of tissue small. Paired sartorius, semitendinosus, iliofibularis, tibialis anticus, and, in small frogs, peroneus muscles from two frogs were used in each experiment. Comparisons were made between paired muscles wherever possible. The dissection was made the evening before the experiment and the muscles kept in oxygenated Ringer-phosphate solution (pH 7.4) at 4 to 5°C. overnight. By this method of preparation muscles are secured whose resting respiration remains remarkably constant (at 30 ± 5 $\overline{mm^3}$/gm/hr.) from experiment to experiment as well as with time during a single experiment. All data on the resting respiration were taken under these conditions and *do not* refer to the respiration of recently dissected, albeit "unstimulated," muscles which are usually burning in part metabolites produced during the dissection.

The muscles were blotted quickly on filter paper, weighed on a torsion balance, and the rates of respiration calculated as $\overline{mm^3}$/gram wet weight/hour. The thermostat temperature was approximately 23°C. regulated to 0.05°C. The gas space contained air (cf. Stannard, 1939a).

Stimulation of the oxygen consumption was produced by 0.04 per cent caffeine in Ringer-phosphate solution since this treatment produces a high and constant rate of respiration which facilitates reasonably accurate calculations of the inhibitions produced. This method of "stimulation" may involve some error not appreciated at present but there is no evidence that increases in the oxygen consumption of frog muscle brought about by this means involve processes differing qualitatively from those produced by electrical stimulation. Reference is made to the publications cited for further details regarding experimental technique.

Two general procedures were used: 1. Measurements were made for a control period of one hour, one member of each muscle pair being in Ringer-phosphate solution, the other member in caffeine-Ringer-phosphate. The inhibitor was then tipped after the initial control period. Frequently one group of muscles did not receive any inhibitor, these acting as continuous controls to check on the constancy of the rate with time. 2. The muscles were treated first with the inhibitor and after a suitable period caffeine was added in appropriate concentration to muscle groups containing one member of each pair of muscles. The remaining muscles continued in the presence of inhibitor alone. There was no essential difference in the results obtained by the two methods if interpreted on the basis of elapsed time in the presence of the inhibitor. The tabular data include both types of experiment.

RESULTS. All of the data are presented as average per cent changes of either the resting respiration or of the extra oxygen consumption due to caffeine. The latter (rate in caffeine without inhibitor minus resting rate = caffeine increment) was not identical in each experiment while the resting rate, as stated above, varied only slightly. The average increment due to caffeine was 125 $\overline{mm}^3$/gm/hr. but varied from 56 to 200 $\overline{mm}^3$/gm/hr. in different experiments. This fact subjects the per cent inhibition to some variation but expression of the results in terms of absolute rates leads to no essential difference in interpretation.[2]

It will be noted that in many instances inhibitions of more than one hundred per cent of the caffeine increment are recorded. This means, obviously, that there was some fall in rate below the original resting level. In general these do not indicate significant effects on the resting respiration.

1. *Sodium fluoride.* The primary action of NaF on glycolysis and indirectly on respiration appears to be inhibition of the conversion of phosphoglyceric acid to pyruvic acid, probably by preventing dephosphorylation (Lipmann, 1928; cf. Cori and Cori, 1941). It has no direct action on cytochrome oxidase (Keilin and Hartree, 1939). The concentrations of fluoride used in the present study were sufficient to prevent dephosphorylation. Yet, as shown in table 1, the resting respiration was not significantly retarded except at the highest concentration in the third hour of exposure. In contrast, as shown in the table, the increment in O_2 uptake due to caffeine is quite sensitive to fluoride, 100 mM fluoride bringing about complete inhibition by the second hour. At a concentration of 10 mM the difference in sensitivity is marked. Furthermore added caffeine was unable to bring about any increase in the rate of respiration after an hour in fluoride (100 mM) although no sign of inhibition of the resting respiration had appeared.

The muscles lost their irritability with or without caffeine at 100 and 170 mM/liter NaF; went into rigor at the end of three hours at the latter but not at the former concentration. At 10 mM/liter irritability was not lost in three hours regardless of the presence or absence of caffeine although the increment in respiration due to caffeine was reduced by 72 per cent. It is interesting to note that resting muscles can apparently lose their irritability and pass into rigor in the presence of fluoride without the usual increase in oxygen consumption associated with the non-irritable state (cf. Fenn, 1930). Apparently these increases in respiration, like those due to caffeine, are inhibited by fluoride.

2. *Sodium arsenite.* As illustrated in table 1 the respiration of caffeinized muscle is much more sensitive to arsenite than is that of resting muscle. Even at the high concentration of 20 mM/liter (one to three mM/liter is

[2] Because of experimental variations changes of less than twenty per cent are not considered significant.

usually sufficient to bring about complete arrest of respiration (Banga, Schneider, and Szent-Györgyi, 1931)) the resting respiration was reduced by only 20 per cent in the time taken to produce 108 per cent inhibition of the caffeine excess. With lower concentrations the effect of caffeine could be largely eliminated without appreciable effect on the resting respiration (except for one experiment at 1 mM/liter NaF). However it

TABLE 1

The action of sodium arsenite, sodium fluoride and sodium pyrophosphate

CONCENTRATION	NUMBER OF EXPERIMENTS	PER CENT CHANGE					
		Resting respiration			Caffeine increment		
		1st hour	2nd hour	3rd hour	1st hour	2nd hour	3rd hour
Sodium fluoride							
mM/liter							
170	1	38	−8	−30	−80	−128	
100	3	66	7(2)*	2(2)*	−41	−97	−107(2)*
10	1	50	47		*	−72	−72
Sodium arsenite							
20	1	−22	−20	−37	−85	−108	−118
10	4	−14	−5	−7(1)	−84	−107	−114
3	3	−8(2)	−3(2)	46(1)	−46	−84	−106
2	3	−14(2)	−1(2)	−20(1)	−22	−81	−105
1	3	−4	−21(1)		*	−89	−98
0.1	2	8			0(1)	−40	−48(1)
Sodium pyrophosphate							
36	1				45	18	
33	1	67	41				
20	1	31	30		50	−5	

* In certain experiments caffeine was tipped onto the muscles after one or two hours in the presence of the inhibitor. Thus some experiments did not run for the full three hours under the same conditions. Figures in parentheses indicate the number of experiments contributing the data where column two does not apply.

will be noted that the resting respiration was more apt to be decreased slightly by arsenite than by fluoride.

At concentrations of 10 and 20 mM/liter the muscles were in rigor at the end of the experiments either with or without caffeine. At 1, 2 and 3 mM/liter irritability was frequently lost in the presence of caffeine but seldom lost with arsenite alone. At 0.1 mM/liter irritability was maintained under both conditions. Apparently arsenite, like fluoride, prevents the increase in oxygen consumption associated with the development of non-irritability and rigor.

The mode of action of arsenite is not as well understood as that of fluoride (cf. Elvehjem *et al.*, 1939) and there has been relatively little recent work bearing on the problem. The consensus places the locus of action nearer the points of "substrate activation" than the oxidases and there is no evidence of direct action on the cytochrome-cytochrome oxidase system (Szent-Györgyi, 1930; Collett, Rheinberger, and Little, 1933; Schmitt and Skow, 1935).

Banga *et al.* (1931) found arsenite-sensitive and arsenite-stable fractions in the respiration of pig heart muscle. A rather extensive analysis of the two fractions was made in Szent-Györgyi's laboratory but, to date, no specific correlation of their data with those reported here has been possible.

3. *Sodium pyrophosphate.* As illustrated in table 1 pyrophosphate, even at relatively high concentrations, did not inhibit the respiration of either resting or caffeinized frog muscle. The muscles remained irritable and showed no evidence of any change after two hours in pyrophosphate.

Keilin (1929) found that pyrophosphate, while an effective inhibitor of certain iron catalyzed reactions, did not inhibit cytochrome oxidase. Dixon and Elliott (1929) reported 87 per cent inhibition of the respiration of rabbit skeletal muscle and 67 per cent inhibition of rat muscle respiration by M/30 pyrophosphate. The action of pyrophosphate on dehydrogenases is still not completely established (Stotz and Hastings, 1937).

The reason for the discrepancy between my results with frog muscle and those of Dixon and Elliott with mammalian muscle preparations is not clear. It should be noted that the frog muscles were practically intact and that Dixon and Elliott found that intact yeast was insensitive to pyrophosphate. In addition rat liver was much less sensitive than muscle even though both preparations presented cut-up tissues. These phenomena may represent real differences in enzyme systems or only differences in permeability to the inhibitor. Further information would be desirable on this point but it was not considered expedient to digress from the use of intact tissues in the present study.

4. *Hydroxylamine.* This inhibitor was found by Stannard (1940) to prevent effectively the oxidation of carbon monoxide by frog muscle without decreasing the resting respiration. In an earlier summary (Stannard, 1939b) a few experiments were quoted which indicated that NH_2OH might also prevent the extra respiration due to caffeine (though not as effectively as the oxidation of CO). In fact it was hoped that this inhibitor might present certain advantages over azide since it acts on cytochrome oxidase *in vitro* in the same manner (Keilin and Hartree, 1939), and does not cause the slight contracture of muscle brought about by azide. More complete data with this substance are presented in table 2. No really marked inhibition of the resting respiration was noted except in the third hour of one experiment at 20 mM/liter. Fairly marked inhibitions of the caffeine excess were found with regularity.

The inhibition is rather slow in developing and incomplete when compared with the action of azide or cyanide. This may indicate slow penetration of the reagent or slow destruction of the active group of some enzyme. The concentrations necessary for appreciable action on caffeinized muscle are considerably higher than those required for inhibition of catalase activity even *in vivo* (Blaschko, 1935). Therefore hydrogen peroxide may have accumulated during these experiments. However, it is improbable that there was a general destruction of all cellular processes (as by H_2O_2) since the resting respiration was unaffected and, except at the highest concentration, the muscles were fully irritable even after the development of marked inhibition of the caffeine increment.

TABLE 2

The effect of hydroxylamine on resting and caffeinized muscle

CONCENTRATION	NUMBER OF EXPERIMENTS	PER CENT CHANGE		
		First hour	Second hour	Third hour
Resting respiration				
mM/liter				
20	2	9	−18	−44(1)*
10	1	−33	−26	
4.5	4	26	8(3)	20(1)
2.0	8	4	−2(5)	2(4)
0.4	2	3	11(1)	
0.04	2	−6	1	
Caffeine increment				
20	2	−59	−88	−104(1)
10	2	−76	−85	
5	1	−58	−68	
2	3	−39	−52	−62

* See footnote to table 1.

5. "*Copper inhibitors.*" In view of the presence of definite copper compounds in the animal body (Mann and Keilin, 1938–39), and recent speculations on the possibility that cytochrome oxidase itself is a copper-protein compound (*cf.* Stern, 1940, p. 3) a study of the action of the so-called copper inhibitors on the two types of respiration found in frog muscle was of interest. Thiourea, sodium diethyl dithiocarbamate, and potassium ethyl xanthate were chosen, and since there was evidence of instability of these compounds in Ringer's solution the reagents were prepared fresh just before each use rather than a few hours previous to the experiment as practiced with the reagents discussed above.

The results are summarized in table 3. Thiourea had relatively little effect on either the resting respiration or the caffeine excess. However, at

sufficient concentration, diethyl dithiocarbamate and potassium ethyl xanthate, if given an hour or more to act, brought about marked decreases in the rate of respiration of caffeinized muscle (but never to the level of resting muscle). One experiment with salicylaldoxime indicated similar results. As with the other inhibitors the resting respiration was not decreased appreciably. In fact with potassium ethyl xanthate there was marked stimulation of the resting respiration.

TABLE 3

The effect of "copper inhibitors" on the respiration of resting and caffeinized muscle

CONCENTRATION	NUMBER OF EXPERIMENTS	PER CENT CHANGE					
		Resting respiration			Caffeine increment		
		1st hour	2nd hour	3rd hour	1st hour	2nd hour	3rd hour
Thiourea							
mM/liter							
220	2				−1	20	13
200	2	5			−7	16	10
4	2	10	6		11(1)*	−15(1)*	−18(1)*
2	1	12	10		−8	5	22
Diethyl-dithio carbamate							
40	2	†	−14		†	−86(1)	
20	2	30	3	−45(1)	†	−85(1)	
4	2	11	23	32(1)	6	−59	−65(1)
2	2	16	4	15(1)	30	−17	−60(1)
Potassium ethyl xanthate							
230	2				7	−48	
200	2	435	310				−60‡
30	1	114	79		−22	−51	−70
20	1	330	154		−15	−41	−72

* See footnote to table 1.

† Complicated by positive pressure. See text.

‡ Caffeine tipped at third hour; muscles already in contracture.

While the present experiments were in progress Graubard (1941) published data on the action of cyanide, diethyl dithiocarbamate, salicylaldoxime, thiourea, and similar substances on the oxidation of p-phenylenediamine by copper and by a cytochrome oxidase preparation obtained from rabbit uterine tissue. In addition he determined the effect of these inhibitors on the respiration of rat and rabbit uteri. Graubard found that salicylaldoxime and diethyl dithiocarbamate were effective inhibitors of O_2 uptake in each case, while thiourea was relatively weak in its action. There appears to be general agreement between these data and those for

frog muscle presented in table 3 except that frog muscle presents a fraction of respiration insensitive to the copper inhibitors while Graubard's curves show complete inhibition of the respiration of rat uteri, at least by salicylaldoxime. Graubard does not state whether or not the inhibitions progressed with time as is the case with frog muscle. The effective concentrations appear to be approximately the same in both studies.

The data with diethyl dithiocarbamate were complicated at the higher concentrations, especially during the first hour, by the production of a positive pressure in the manometers (cf. also Graubard, 1941). If this occurred also at lower concentrations the inhibitions observed might be spurious in that they represented only the algebraic sum of normal oxygen consumption and the positive pressures produced. However control experiments with resting muscle did not indicate the presence of sufficient positive pressure to complicate the readings with caffeine below 20 mM/liter or after the first hour. There was no indication that the presence of caffeine increased the positive pressure produced at a given concentration of inhibitor. In addition no such difficulty was encountered with potassium ethyl xanthate which probably inhibits respiration in the same manner.

Diethyl dithiocarbamate and potassium ethyl xanthate usually produced non-irritable muscles in the time and at the concentrations required to inhibit appreciably the caffeine increment in respiration. Resting muscle lost its irritability only at the highest concentration of each substance while with caffeine, rigor occurred at this concentration. Thus the combination of inhibitor and caffeine produced rigor more readily than either alone. However, even with caffeine, inhibition of the oxygen consumption did not appear to be directly related to changes in irritability or contractility.

6. *Contracture.* Potassium ethyl xanthate was found by means of kymographic experiments to produce a small but rapid contracture of the isolated sartorius muscle. None of the other substances used in these experiments produced appreciable changes in resting length (under 20 grams, tension) until rigor supervened, although changes of 5 per cent or less were noted occasionally. The contracture produced by potassium ethyl xanthate probably accounts for the marked rise in O_2 consumption seen when resting muscle was treated with this substance (cf. Fenn, 1931). The situation in this case is probably similar to that seen with azide (Stannard, 1939a).

7. *Reversibility.* The inhibitions produced by hydroxylamine were reversible over a fairly wide range of concentrations and times of exposure. Those produced by fluoride and arsenite were not clearly reversible except after exposures of less than one hour and/or at low concentrations (10 mM/liter). No information was obtained concerning the reversibility of

the effects brought about by the "copper inhibitors." It is clear that the main arguments of the present paper are not materially affected by whether or not inhibition was produced by destruction of a certain enzyme or by formation of a reversible enzyme-inhibitor complex, since the fact remains that the action involves only one fraction of the total respiration.

DISCUSSION AND CONCLUSIONS. Since members of the methylxanthine group have been shown to increase cellular permeability in certain instances (Fröhlich, 1928) it might be thought that the data presented here indicate simply that caffeine increased the permeability of the muscle cells to the inhibitors. Therefore it is emphasized again, as in the case of azide (Stannard, 1939a), that the maximum effect brought about by the action of these inhibitors on caffeinized muscle brings the rate of respiration to approximately that of the original resting state (except when drastic procedures are used). In addition penetration of the reagents even into resting muscles is established by the frequent changes in irritability occurring despite constancy of the resting respiration and by the experiments in which the addition of inhibitors to resting muscle prevented any subsequent increases in respiration on the addition of caffeine.

The action of the "copper inhibitors" is of considerable interest. The presence of copper as the active metal in several plant oxidases is now well established. Except for Graubard's study (op. cit.) on uterine tissue and the cytochrome oxidase prepared therefrom scanty evidence is available indicating the presence of copper in oxidases from animal sources. The fact that certain of the compounds employed inhibit a major fraction of frog muscle respiration cannot be taken as proof that the reaction involves a copper-containing enzyme for, while it is true that these reagents combine rapidly and with minute quantities of copper, they also react with other metals under appropriate conditions. In comparison with the rapid and complete inhibitions obtained with plant oxidases the action of diethyl dithiocarbamate and of potassium ethyl xanthate on the respiration of frog muscle seems slow and relatively weak. However, a more complete study of these reactions in frog muscle is contemplated since the evidence for the participation of copper, while circumstantial, seems worthy of further consideration. It should be noted that the fraction of respiration affected is that fraction which utilizes the cytochrome-cytochrome oxidase system.

The data presented above combined with the results of previous investigations indicate that the activity respiration (caffeine increment) of frog muscle is sensitive to azide, cyanide, and hydroxylamine, all of which prevent the normal activity of cytochrome oxidase (Keilin and Hartree, 1939). Certain of the "copper inhibitors" likewise prevent or retard the activity respiration. In addition sodium fluoride and sodium arsenite,

whose locus of action is probably on certain dehydrogenases, inhibit or prevent the increases in respiration associated with stimulation by caffeine. In contrast none of the inhibitors tested, except cyanide, exert any specific effect on the resting respiration. Recently Stern and Fisher (1941) have found that ethyl urethane, phenobarbital, and chlorobutanol, all acting on dehydrogenases presumably, bring about strong inhibition of the activity oxygen consumption of frog muscle with relatively little effect on the resting respiration. Thus it appears that the resting respiration does not utilize the cytochrome-cytochrome oxidase system nor the generally known dehydrogenase systems inhibited by fluoride, arsenite, ethyl urethane, etc., these systems functioning only in activity.

Other recent instances of qualitative differences between the metabolism of tissues at rest and in activity are presented by Korr (1941), and Fisher (1941) has suggested a mechanism of narcosis based on the greater sensitivity of the processes responsible for specific cellular functions (growth, light production, contraction, etc.) to the action of the drug employed. If the present trend continues a closer correlation of function with metabolism may be possible.

SUMMARY

1. The respiration of resting frog muscle was not inhibited specifically by fluoride, arsenite, hydroxylamine, pyrophosphate, thiourea, diethyl dithiocarbamate, or potassium ethyl xanthate.

2. Increases in oxygen consumption brought about by the presence of 0.04 per cent caffeine (activity respiration) were inhibited or prevented by suitable concentrations of fluoride, arsenite, hydroxylamine, diethyl dithiocarbamate, and potassium ethyl xanthate. The rate of respiration after the maximum effect of the inhibitors was exerted approximated that of resting muscle.

3. The cases where neither the resting respiration nor the increment in respiration due to caffeine were inhibited may represent true lack of sensitivity of the enzyme systems or impermeability of the muscles to these reagents.

4. It is proposed that the activity respiration of frog muscle differs from the resting respiration not only in utilizing the cytochrome-cytochrome oxidase system, but in utilizing different dehydrogenases as well. Actually the two systems may be completely independent.

5. Sensitivity of the activity respiration to compounds thought to inhibit copper catalyses (diethyl dithiocarbamate and potassium ethyl xanthate) may indicate the presence of a copper-containing enzyme in frog muscle, but further experiments are required to establish this point.

6. Resting muscle could frequently lose its irritability or even go into rigor in the presence of the inhibitors without any change in the rate of respiration.

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RESPIRATION AND GLYCOLYSIS OF BONE MARROW OF RABBITS EXPOSED TO LOWERED OXYGEN TENSION

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That the nucleated red blood cells in rabbit bone marrow are characterized metabolically by relatively active respiratory, rather than glycolytic, processes has been shown by experiments recently reported in this Journal (1). Since it is well known that exposure of animals or man to lowered oxygen tension stimulates the bone marrow to increased hematopoietic activity, the question at once arises whether this characteristic oxidative type of metabolism of the erythroid cells in the bone marrow is altered under these conditions. It has been shown by Argyll Campbell (2) that when rabbits are exposed to lowered atmospheric pressure, not only is the oxygen tension in the tissues reduced below the normal level, but it remains reduced despite the ensuing polycythemia. One might reasonably inquire whether, under these circumstances, the erythroid cells develop more active glycolytic mechanisms which might enable them to grow and multiply more readily in the environment of lowered oxygen tension. The present paper describes experiments by which this problem has been investigated. A series of rabbits were exposed to lowered oxygen tension for various periods of time and changes in the rates of respiration and glycolysis of the bone marrow followed in the Warburg apparatus. An attempt has also been made to determine whether these metabolic changes are controlled by humoral or neural mechanisms.

I. METHODS. Male New Zealand White rabbits weighing about 2 kilos were placed in a tank[2] through which air was drawn under a pressure of 410 mm. Hg, corresponding to atmospheric pressure at an altitude of about 16,000 feet. They were removed from the tank for several hours a day to be fed and watered. After several days, reticulocytes appeared in the circulating blood in increasing numbers, followed rapidly by increases in the red blood cell and hemoglobin levels. Figure 1, which is self-explan-

[1] This work was done during the tenure of a Lewis Cass Ledyard Jr. Fellowship, New York Hospital, 1940–41.

[2] Kindly loaned by the Department of Biology, Washington Square College, New York University.

atory, shows these changes in two illustrative cases. Leucocyte counts are not shown because they were not affected by the exposure to lowered atmospheric pressure except in 4 animals which, after exposures of about a week, developed an enteritis manifested by diarrhea. In these, a leucocytosis occurred along with the polycythemia; they will be discussed separately below. The graphs are terminated by the animals being sacrificed for the metabolic and morphologic studies of the bone marrow.

For the metabolic studies, slices of the femoral bone marrow of uniform thickness (0.5 mm.) were placed in Warburg vessels containing 2 ml. of autogenous serum. The methods used in preparing these slices and in measuring the rates of respiration and anaerobic glycolysis in serum, and "apparent aerobic glycolysis" in Ringer-bicarbonate-glucose medium have already been described (3, 4). Differential cell counts were made on smears of each marrow stained with Wright-Giemsa.

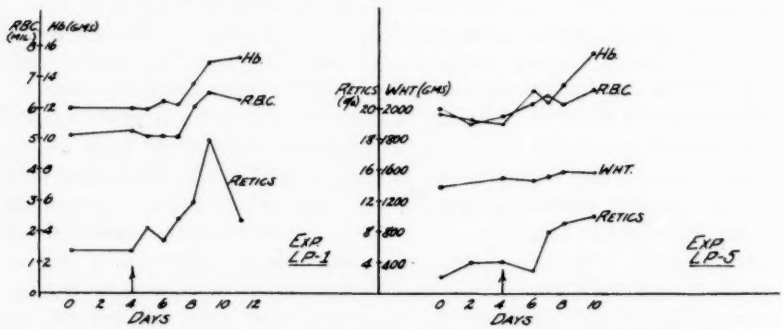


Fig. 1. Blood counts of two of the animals exposed to lowered atmospheric pressure. Arrow indicates beginning of the exposure.

II. Results. The results of 25 experiments are summarized in table 1, in which the marrows are arranged in the order of increasing erythroid hyperplasia. It will be seen that this order is not the same as that of increasing periods of exposure to lowered oxygen tension, owing probably to the variability of the differential counts at the start of the exposures as well as to differences in the response of the individual animals. Also, in the marrows of the 4 animals which developed a diarrhea and leucocytosis, hyperplasia of both the myeloid and erythroid elements was found, so that they appear earlier in the series than they would had this complication not arisen.

In the last column, the ratios of anaerobic glycolysis/respiration ($Q_G^{N_2}/Q_{O_2}$) are given. It has been noted in an earlier study (1) that these ratios constitute a more dependable index of the metabolic activity of the marrow than either the $Q_G^{N_2}$ or the Q_{O_2} alone. It is seen from table 1 that, in gen-

eral, these ratios decrease as the proportion of erythroid cells increases, a relationship shown more clearly when the data are plotted in graphic form in figure 2. The crosses in this figure indicate normal control animals and the circles, the 25 marrows listed in table 1, the solid circles representing the animals which developed the enteritis. The dotted line, which appears to be drawn with reference to these points is, in fact, obtained from data given in the previous publication (1) in which the erythroid hyperplasia was induced by bleeding and by injections of phenylhydrazine. Since the present data fit this curve within the limits of experimental error, it is clear that the respiration and glycolysis of the marrow are determined by the types of cells present rather than by the means used to induce the erythroid

TABLE 1

Cell counts and glycolysis: respiration ratio in marrows from animals exposed to lowered atmospheric pressure

EXPERIMENT NO.	DAYS IN TANK	PER CENT ERYTHROID CELLS	RATIO $Q_G^{N_2}/Q_{O_2}$	EXPERIMENT	DAYS IN TANK	PER CENT ERYTHROID CELLS	RATIO $Q_G^{N_2}/Q_{O_2}$
25	1	51	2.32	19	2	71	1.38
8*	9	57	1.81	23	8	71	1.49
9*	7	60	2.02	20	2	73	1.56
13	2	62	1.73	5	6	74	1.52
26	1	63	1.65	22	3	74	1.48
18	2	65	1.58	11	2	75	1.48
12	1	66	1.83	3	4	79	1.28
21	2	66	1.65	14	3	79	1.36
17	3	67	1.56	4	4	81	1.33
7*	7	69	1.31	6	4	81	1.19
16	2	70	1.60	1	7	85	1.22
10*	5	71	1.73	24	4	88	1.24
15	1	71	1.31				

* Animal had diarrhea.

hyperplasia. It is true, of course, that all three procedures,—bleeding, injections of phenylhydrazine and exposure to lowered atmospheric pressure—have the common result of lowering the oxygen tension in the marrow. But, as has already been mentioned, exposure to lowered atmospheric pressure causes a more profound and lasting reduction in oxygen tension in the tissues than either bleeding or hemolytic agents. The above experiments show, however, that the characteristic oxidative type of metabolism of the erythroid cells (indicated by low values of the glycolysis/respiration ratio) persists despite prolonged exposure to an environment of lowered oxygen tension.

It is obviously important to inquire whether the low glycolysis/respira-

tion ratio characteristic of these erythroid marrows is due principally to an increase in the rate of respiration or to a decrease in the rate of glycolysis. The data relative to this point are shown in graphic form in figure 3 A and B, in which the Q_{O_2} and the $Q_G^{N_2}$, respectively, are plotted against the per cent of erythroid cells. The crosses in these figures represent corresponding data for normal marrows taken from the previous publication. The Q-values are based on fat-free dry weights calculated, as previously explained (4), from nitrogen determinations. It is seen that the greater part of the decrease in the glycolysis/respiration ratio is due to

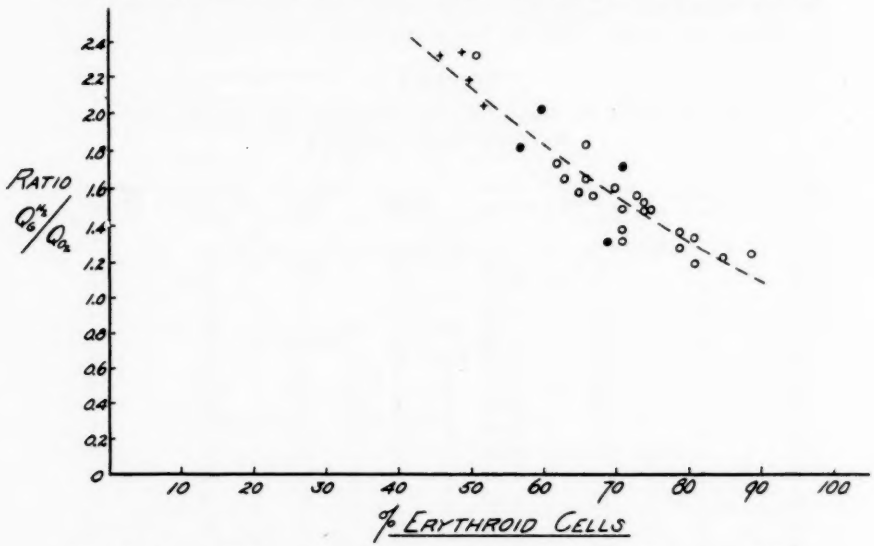
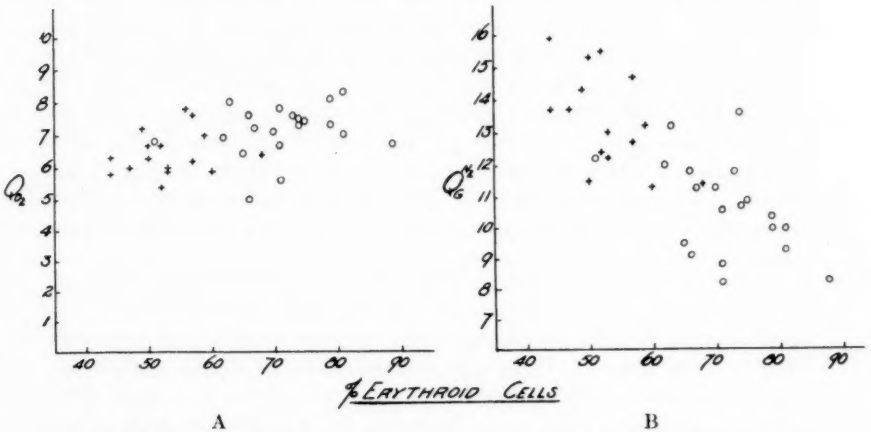


Fig. 2. Crosses indicate normal control animals, circles the 25 marrows listed in table 1. Solid circles indicate the animals which developed enteritis. The dotted line is drawn with reference to similar points in a previous publication (1), in which the erythroid hyperplasia was induced by other means.

a decrease in anaerobic glycolysis[3], rather than to an increase in the rate of respiration. In fact, owing to the spread in the data, it is not clear whether there is any significant increase in the Q_{O_2} of the marrows of the animals exposed to lowered atmospheric pressure. The mean Q_{O_2} of 20 of these hyperplastic marrows is 7.12 with a P.E. of $\pm$ 0.12; that of 17 normal marrows is 6.47 $\pm$ 0.15. The average increase in Q_{O_2} of 0.65 (a 10 per cent increase over the normal) is 3.4 times the P.E. of the difference in the means and hence has statistical significance, but the physiological

[3] Aerobic glycolysis is also reduced in these erythroid marrows. The $Q_G^{O_2}$ averaged 1.1 as compared with 2.7 for normal marrows.

significance of this finding is less easily stated. Possible interpretations are 1, that the erythroid cells have slightly higher rates of respiration than myeloid cells; 2, that hyperplastic marrows are "stimulated" marrows and hence have higher rates of respiration than "resting" marrows; 3, that the change is due to an increased proportion of immature cells, or 4, to a decreased proportion of metabolically inactive but nitrogen-containing connective tissue in these hyperplastic marrows; 5, that the nitrogen content does not represent the same fraction of the fat-free dry weight in hyperplastic as in normal marrows, or finally 6, a combination of these possibilities. Because hyperplastic *myeloid* marrows show the same changes



A B

Fig. 3-A. Relationship between rate of respiration and extent of erythroid hyperplasia.

Fig. 3-B. Relationship between rate of anaerobic glycolysis and extent of erythroid hyperplasia.

Crosses represent corresponding data for normal control animals, taken from a previous publication (1); circles the marrows listed in table 1.

in $Q_G^{N_2}$ and Q_{O_2} but in the opposite sense (1) and because there is no correlation between $Q_G^{N_2}$ or Q_{O_2} and counts of immature cells, the interpretation of a similar finding in the earlier paper was that erythroid cells have slightly higher rates of respiration and lower rates of anaerobic glycolysis than myeloid cells. This conclusion is still valid and, indeed, is confirmed by the present studies, but the other factors must be kept in mind as perhaps playing an accessory rôle until they are excluded by experimental evidence.

III. *Combined effects of exposure in vivo and in vitro to lowered oxygen tension.* In the above paragraphs it has been shown that the respiration, in pure oxygen, and the glycolysis, both in oxygen and in nitrogen, of the bone marrow of rabbits exposed to lowered oxygen tension is the same as

that of marrows in which a similar degree of erythroid hyperplasia has been induced by other means. The possibility still remains that at intermediate (lowered) oxygen tensions, differences in the metabolic behavior between the two groups of marrows might be observed.

When either normal marrows, or marrows in which an erythroid hyperplasia has been induced by bleeding or by injections of phenylhydrazine, are subjected in the Warburg apparatus to a series of lowered oxygen tensions, the rate of respiration is decreased and that of glycolysis is increased in reciprocal fashion. A detailed report of the experiments upon which this statement is based, and a discussion of its significance, will be given in a separate publication on the Pasteur effect in bone marrow, now in preparation. At this time, however, it seems desirable to mention the results obtained in four similar experiments, in which marrows from animals which had been in the low-pressure tank and which contained from 60 to 75 per cent erythroid cells were exposed in the Warburg apparatus to oxygen tensions ranging from 5 to 40 per cent oxygen. All showed the same reciprocal relationship between respiration and glycolysis, so that no difference in response to exposure *in vitro* to lowered oxygen tension could be demonstrated following previous exposure *in vivo* to lowered atmospheric pressure.

IV. *Serum factors.* Gordon and Dubin (5) have called attention to reports in the literature of the alleged presence of a hematopoietic stimulant in the serum of rabbits either rendered anemic or subjected to low pressures. Their own experiments failed to confirm the existence of such a substance, but the possibility still remained that normal bone marrow metabolism might be affected by the serum of animals being subjected to the hematopoietic stimulus of lowered atmospheric pressure. Also, the rate of respiration and glycolysis of the bone marrow of the "low pressure" animals might itself be dependent upon the presence of substances contained in the serum. These possibilities were investigated by the experiments summarized in table 2. As control experiments, the effect of normal homologous serum on the respiration and anaerobic glycolysis of two normal marrows was first studied (group I). The second of these marrows fortuitously had an unusually high percentage (65 per cent) of erythroid cells and consequently a low $Q_G^{N_2}/Q_{O_2}$ ratio (1.60). In both instances, the Q-values measured with the marrow slices suspended in homologous serum were about 12 per cent lower than in autogenous serum. Respiration and glycolysis were similarly affected, so that the $Q_G^{N_2}/Q_{O_2}$ ratios were essentially the same in the two media.

In group II similar measurements were made on three normal marrows, comparing their respiration and glycolysis in autogenous serum with that in serum freshly removed from animals in the low-pressure tank. Here again the characteristic glycolysis/respiration ratios were found not to be affected

by the "low-pressure" serum and the Q-values were slightly depressed. Hence Gordon and Dubin's negative findings are extended by the failure to obtain evidence for a stimulating effect of "low-pressure" serum on the metabolism of normal bone marrow.

In group III, the reciprocal experiments were performed in which the effect of the normal serum on the "low-pressure" marrows was observed. The effects are within the limits of experimental error and it is clear that the low glycolysis/respiration ratios characteristic of erythroid marrows are not dependent, at least over a period of several hours, on a substance or substances which may be present in the serum. Bone marrow function

TABLE 2

Marrow respiration and glycolysis in serum from other rabbits

GROUP	MARROW	PER CENT ERYTHROID CELLS	FOREIGN SERUM	CHANGE FROM METABOLISM IN OWN SERUM		RATIO $Q_G^{N_2}/Q_{O_2}$	
				Q_{O_2}	$Q_G^{N_2}$	In own serum	In foreign serum
				per cent	*per cent*		
I. Normal marrows in sera of other normal rabbits	1	50	Normal	−14	−7	2.18	2.35
	2	65	Normal	−12	−11	1.60	1.61
II. Normal marrow in sera of "low pressure" rabbits	3	49	LP-18	−10	−14	2.33	2.24
	4	52	LP-19	−3	−5	2.04	2.00
	5	46	LP-20	−1	−4	2.32	2.43
Average						2.23	2.22
III. "Low pressure" marrow in serum of normal rabbits	LP-18	65	3	+4	+6	1.58	1.60
	LP-19	71	4	+1	+7	1.38	1.45
	LP-20	73	5	−9	−6	1.56	1.61
Average						1.51	1.55

may be under the influence of humoral agents (6, 7, 8), but the experiments just described lend no support to the view that the stimulating effect of lowered oxygen tensions is mediated through such a mechanism.

V. *Possible rôle of the peripheral nervous system in mediating the effects of lowered oxygen tension.* These experiments were designed to test the possibility that lowered oxygen tension acts by stimulating chemoreceptors in the nervous system and that impulses might then be relayed via the peripheral nervous system to the bone marrow, there evoking increased hematopoietic activity. The well-known sensitivity of the nervous system to oxygen lack, and the demonstration by Latner (9) that removal of the

carotid sinuses and denervation of the aortic arch in rabbits induced an anemia which could not be accounted for by loss of blood at operation lent some support to the first part of this concept. Also, innervation of the blood vessels in the bone marrow has been described (10), and it is even possible (11, 12) that the marrow cells themselves may be innervated. Rohr (13) has cited other evidence in favor of a neural mechanism controlling bone marrow function.

The possibility that the effects of lowered oxygen tension might be mediated via the peripheral nervous system was investigated by sectioning the femoral and sciatic nerves in the right thigh of a group of rabbits, thus denervating that limb below the knee. About a week after operation, when physical examination and temperature measurements using the opposite leg as control indicated that the denervation was complete, three of the animals were placed in the low-pressure tank, another serving as control. After a further interval of 4 to 7 days, the animals were sacrificed

TABLE 3

Effect of denervation on marrow response to low pressure

EXPERIMENT NUMBER	DAYS IN TANK	PER CENT RETICULOCYTES		PER CENT ERYTHROID CELLS IN MARROW			
		At beginning	At end	Right (denervated)		Left	
				Mature	Immature	Mature	Immature
H-6*	0	6.4	7.0	51	12	51	13
H-5	4	4.2	15.7	75	22	74	28
H-2	6	3.8	10.0	83	19	84	20
H-4	7	3.9	12.8	77	22	75	22

* Control.

and differential cell counts made on smears of the tibial marrow of the right and left (control) limb of each animal. The results are indicated in table 3.

It is seen that both in the control animal, which did not develop an erythroid hyperplasia, and in those which were subjected to lowered atmospheric pressure and consequently did, the counts in the two limbs were the same within the limits of experimental error. The criterion of "maturity" of the nucleated erythroid cells in the marrow was the same as that used in the previous publication,—any cell which contained hemoglobin was counted as "mature". Non-nucleated erythrocytes were not counted. It is clear that the response of the bone marrow to lowered atmospheric pressure is not dependent upon an intact peripheral (including autonomic) innervation.

VI. Discussion. The erythroid hyperplasia resulting from the exposure of rabbits to lowered oxygen tension has been shown to be accompanied by a small but significant increase in the rate of respiration and a

much larger fall in the rate of anaerobic and aerobic glycolysis when the Q-values are based on nitrogen determinations. In rats, Schultze (14) has made the important observation that the cytochrome oxidase activity of the bone marrow of normal adult animals is increased to the level of that found in immature animals by 48 hours' exposure to lowered oxygen tension. This increased activity, however, is based on total dry weight rather than on nitrogen determinations, and since histological examinations were not made, it is not clear whether the increased enzymatic activity is due to replacement of fat by erythroid tissue, to a higher enzyme content of the erythroid as compared to the myeloid cells, to an increased content of the erythroid cells *per se* or, as seems most likely, to a combination of these factors. Formulation of the relationship between such enzyme studies and our own kinetic studies would be of considerable interest, but must await the results of experiments in which both types of measurement are made on the same tissue under the same experimental conditions. Such studies are now in progress.

The finding in section V, that precisely the same degree of erythroid hyperplasia occurs in denervated as in normal marrows when the animals are exposed to lowered atmospheric pressure, is strong evidence that the hematopoietic activity of the bone marrow is not directly dependent upon impulses carried by the peripheral (including the autonomic) nervous system. This does not preclude the possibility, suggested particularly by the experiments of Davis (15), that vasomotor control of the marrow vessels may exert some subsidiary effect on hematopoietic function, but, on the other hand, the present experiments lend no support to such a concept. Also, the possibility still remains that existence of lowered oxygen tension is first detected by chemoreceptors in the nervous system and that the effects are subsequently relayed to the bone marrow by humoral mechanisms not detected in the acute *in vitro* experiments described in section IV.

SUMMARY AND CONCLUSIONS

1. When rabbits are exposed to atmospheric pressure sufficiently low to produce pronounced polycythemia in the circulating blood and erythroid hyperplasia in the bone marrow, the rate of respiration of the marrow is increased about 10 per cent and that of anaerobic glycolysis is reduced about 30 per cent. These are average rather than maximal figures. Aerobic glycolysis (in pure oxygen) is also markedly reduced.

2. These changes correspond exactly to those found when a similar degree of erythroid hyperplasia is induced by hemorrhage or by injections of phenylhydrazine. Bone marrow respiration and glycolysis therefore appear to be determined by the histological composition of the marrow rather than by the means used to induce these morphological changes.

3. There is no evidence that the glycolytic components of the respiratory systems in the erythroid marrow cells are increased when the cells are formed under the influence of lowered oxygen tension, although the rate of glycolysis may be higher than normal under the conditions of lowered oxygen tension (Pasteur effect).

4. Exposure of rabbits to lowered atmospheric pressure does not alter the response of the bone marrow to exposure *in vitro* to lowered oxygen tension.

5. Serum taken from animals subjected to lowered atmospheric pressure does not stimulate either the respiration or glycolysis of normal marrows.

6. The relatively low glycolysis/respiration ratios characteristic of erythroid marrows are not dependent, at least over a period of several hours, on substances which may be present in the serum.

7. Exposure to lowered atmospheric pressure induces the same degree of erythroid hyperplasia in the marrow of denervated and control limbs. The marrow response is consequently not dependent upon an intact innervation.

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